Exhibit 1

Kniska, Virginia

From: Allaband, Donna <DAllaband@MNAT.com>
Sent: Friday, December 2, 2016 3:53 PM
To: Pivovar, Adam; Allaband, Donna; z/HyperBranch-Integra; 'Filesite'; Grimm, Thomas; Graves, Jon; Kraftschik, Stephen; lbcases; Lockhart, Nicholas; Crenshaw, Stephen; Tigan, Jeremy A.
Subject: HyperBranch/Integra 15-819: Minute Entry re Telephone Conference w/Judge Burke on 12/1/16.

On behalf of Tom Grimm, please see the Docket Text entry below:

The following transaction was entered on 12/2/2016 at 3:41 PM EST and filed on 12/1/2016
Case Name: Integra LifeSciences Corp. et al v. HyperBranch Medical Technology, Inc
Case Number: 1:15-cv-00819-LPS-CJB
Filer:
Document Number: No document attached

Docket Text:
Minute Entry for proceedings held before Judge Christopher J. Burke - Telephone Conference held on 12/1/2016. The Court heard argument from the parties regarding their discovery disputes, (D.I. [200]). The Court granted Defendant's motion for relief and ordered Plaintiffs to provide supplemental responses to Defendant's Interrogatories Nos. 1 and 2 by December 9, 2016. The Court will issue a written order regarding Plaintiffs' disputes. (Court Reporter Valerie Gunning. Clerk: Crawford) Appearances: K. Pascale, C. Roth, R. Altherr for Plaintiffs; T. Grimm and A. Pivovar for Defendant. (mlc)

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Exhibit 2

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

INTEGRA LIFESCIENCES CORP., INTEGRA LIFESCIENCES SALES LLC, CONFLUENT SURGICAL, INC., AND INCEPT LLC, Plaintiffs, v. HYPERBRANCH MEDICAL TECHNOLOGY, INC., Defendant.	C.A. No. 15-819-LPS-CJB

PLAINTIFFS' SUPPLEMENTAL OBJECTIONS AND ANSWERS TO HYPERBRANCH'S INTERROGATORIES NOS. 1 AND 2

Pursuant to Rules 26 and 33 of the Federal Rules of Civil Procedure, the Local Rules for the U.S. District Court for the District of Delaware, and subject to their rights to supplement these objections later in discovery, Plaintiffs Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical, Inc., and Incept LLC (collectively "Plaintiffs," as well as "Integra," "Integra Sales," "Confluent," and "Incept," respectively) hereby provide the following supplemental objections and responses to Defendant HyperBranch Medical Technology's ("HyperBranch") First Set of Interrogatories (Nos. 1 and 2) , including each and every definition, instruction, and interrogatory contained therein (collectively "HyperBranch's First Set of Interrogatories"). The fact that Plaintiffs provide an answer to an interrogatory does not constitute an admission or acknowledgement that the interrogatory is proper, that the answers sought are within the bounds of discovery, or that requests for similar information will be treated in a similar fashion. Plaintiffs do not waive any objection by producing such documents, things, or answers, and Plaintiffs reserve the right to continue investigating these matters, to supplement their objections, and to object to future discovery on the same or related matters. Plaintiffs

further reserve the right to object to the admissibility of any answer produced pursuant to these interrogatories, in whole or in part, on any ground including without limitation materiality, relevance, and privilege.

GENERAL OBJECTIONS

Plaintiffs incorporate by reference their General Objections and Objections to Specific Definitions to HyperBranch's Requests for Production. Each of these General Objections is incorporated into the specific objections set forth below, whether or not separately set forth therein.

1. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks to impose upon Plaintiffs any obligation or responsibility broader than, different from, or in addition to those obligations and requirements mandated by the Federal Rules of Civil Procedure, the Federal Rules of Evidence (collectively, the "Federal Rules"), and the Local Rules for the United States District Court for the District of Delaware (the "Local Rules").

2. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs do not intend to produce such privileged or protected documents or information. To the extent that any document or information which is properly subject to any such privilege or protection is inadvertently produced in connection with an answer to an interrogatory, such inadvertent disclosure is not to be construed as a waiver of such privilege or protection, and such document and information, and all copies thereof, shall be returned to counsel for Plaintiffs, in accordance with Fed. R. Evid. 502(b), Fed. R. Civ. P. 26(b)(5)(B), and any relevant Order entered by the Court. Further,

Plaintiffs will limit their privilege log to pre-lawsuit privileged or protected documents or information, if any exist.

3. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent they contain misstatements of fact and/or inaccurate assumptions. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it is overly broad, unduly burdensome, or oppressive. Plaintiffs further object to each and every definition, instruction, and interrogatory to the extent it calls for information that is irrelevant to any claim or defense in this action.

4. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks information already in the possession, custody, or control of HyperBranch as being overly broad, unduly burdensome, expensive, and inconsistent with the Federal Rules.

5. Plaintiffs object to each and every definition, instruction, and interrogatory as being unduly burdensome to the extent it seeks facts, documents, and/or information that is publicly available, unreasonably cumulative or duplicative, or already known and equally available to HyperBranch.

6. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it is vague, ambiguous, fails to describe the information sought with the required reasonable particularity, or is so unintelligible that Plaintiffs cannot ascertain what information is responsive.

7. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks to impose upon Plaintiffs an obligation to investigate or discover information, materials, or documents from any entity other than Plaintiffs, including, but not limited to, third parties or non-parties.

8. Plaintiffs' agreement to furnish information in response to HyperBranch's Interrogatories shall not be deemed to constitute an admission as to its relevancy, nor is it intended to waive any right to object to its admissibility at trial.

9. Plaintiffs object to each interrogatory that requests "each," "every," or "all" (and to similar overly broad terms) information or documents as overbroad and unduly burdensome. Plaintiffs will undertake a diligent and reasonable investigation to gather information in their possession, custody, or control that is responsive to the non-objectionable portions of each interrogatory.

10. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it contains subparts, is compound and conjunctive, and is otherwise inconsistent with or exceeds the number of interrogatories permitted by any relevant Order entered by the Court. The Court has set a limit of 25 interrogatories for each side. In answering any or all of these Interrogatories or subparts, Plaintiffs do so without waiver of their right to object to and refuse to answer any future Interrogatories on the grounds that such Interrogatories are in excess of the number permitted by the Federal and Local Rules and the Court's Scheduling Order.

11. In addition to these General Objections, Plaintiffs have specific objections as set forth below. By stating these specific objections, Plaintiffs do not waive any of the General Objections that may also be applicable to specific interrogatories.

OBJECTIONS TO SPECIFIC DEFINITIONS

1. Plaintiffs object to the definition of the terms "Plaintiffs," "You," and "Yours" to the extent those terms are overly broad and purport to require Plaintiffs to provide information and/or documents that are not currently within their possession, custody, or control. Plaintiffs object to the definitions of the terms "Plaintiffs," "You," and "Yours" as seeking the disclosure

of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law, in that the definitions specifically cover "attorneys."

2. Plaintiffs object to the definition of "Accused Products" as overbroad, unduly burdensome, and irrelevant to any issue in this matter as "any and all products, activities, services, processes, systems, apparatuses, or things that Plaintiffs accuse of infringing the Asserted Patents in this Action, including Adherus Autospray Dural Sealant, Adherus Dural Sealant, and Adherus Spinal Sealant" include information, products, and/or documents that are not currently within the possession, custody, or control of Plaintiffs. Indeed, this definition explicitly includes documents and things which are in the exclusive control of Defendant and Third Parties.

3. Plaintiffs object to the definition of the term "each" to the extent that the definition purports to impose a meaning broader than the definition provided in the Federal Rules.

4. Plaintiffs object to the definition of "Prior Art" as overbroad, unduly burdensome, and irrelevant to any issue in this matter as "all things, patents, publications, disclosures, sales, or other acts or occurrences included within the broadest meaning of 35 U.S.C. § 102 (or any subpart thereof) and 35 U.S.C. § 103" and "publications, patents, patent applications, inventions by others, uses, sales or offers for sale, and disclosures" purports to require Plaintiffs to provide information and/or documents that are not currently within their possession, custody, or control.

OBJECTIONS AND ANSWERS TO SPECIFIC INTERROGATORIES

INTERROGATORY NO. 1 [9]. On a claim-by-claim basis for each and every claim of the Asserted Patents, identify each individual who You contend contributed to the conception of the invention set forth in each claim, including all supporting facts and evidence of the contribution to the conception of each claim by the identified individual(s) and the dates of such contribution(s).

OBJECTION AND ANSWER TO INTERROGATORY NO. 1 [9]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this interrogatory to the extent it purports to be HyperBranch's first interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's ninth interrogatory. Plaintiffs further object to this interrogatory as being unreasonably cumulative or duplicative, or already known to HyperBranch. *See* Interrogatory No. 1 served by HyperBranch on October 23, 2015. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome in that it requests identification of "all supporting facts and evidence of the contribution to the conception of each claim." Plaintiffs further object to this interrogatory as premature and irrelevant to the extent it is a contention interrogatory that seeks to impose a burden on Plaintiffs to provide a rebuttal position on conception of the inventions claimed in the patents-in-suit prior to the provision of any contention of invalidity of the claims that Defendant is required to provide on November 4, 2016. Validity, including validity of conception and proper inventorship is presumed by the issuance of the patent. Defendant bears the burden of establishing through its

invalidity contentions that there is an issue as to validity that would require Plaintiffs to prove an earlier date of invention or confirm the contribution of a listed inventor to the claims of the patents-in-suit. To date, Defendants validity contentions have not met that burden. Plaintiffs also object to this interrogatory to the extent it calls for legal argument and/or expert testimony, which Plaintiffs may provide, in due course and in accordance with the Court's Scheduling Order.

Subject to and without waiving its objections, Plaintiffs incorporate by reference their response to Interrogatory No. 1 served on November 13, 2015 and all supplements thereto and the Rebuttal Expert Report of Dr. Jimmy Mays and further respond that based on present information Chandrashekhar P. Pathak, Amarpreet S. Sawhney, and Peter G. Edelman contributed to the conception of one or more claims of the '034 Patent, the '406 Patent, the '5,705 Patent, the '566 Patent and the '418 Patent. Plaintiffs further respond that based on present information Amarpreet S. Sawhney, Steven Bennett, and Peter G. Edelman contributed to the conception of one or more claims of the '3,705 Patent. Defendants' present invalidity contentions do not place in dispute the conception or the named inventor's individual contributions to conception of any of the claims. Accordingly, Plaintiffs presently intend to rely on the effective filing date for each of patents-in-suit (including those patents and patent applications to which priority is claimed), including any evidence presented during prosecution of the patents-in-suit (including those patents and patent applications to which priority is claimed), the recitation of the named inventors on the face of each of the patents-in-suit, and the prior sworn deposition testimony (including exhibits used in those depositions) in this matter of the named inventors to identify the dates and individuals contributing to the conception of each of the claims of the patents-in-suit and the prior sworn testimony and multiple expert reports,

rebuttal expert reports, and/or declarations of Dr. Jimmy Mays that have previously been provided in this matter. Plaintiffs further respond that they have produced non-privileged documents pursuant to Federal Rule of Civil Procedure 33(d) (including the patents-in-suit, the patents and applications from which the patents-in-suit claim priority, the prosecution histories of these patents and patent applications, and the laboratory notebooks and the reports summarizing the laboratory work and notebooks of the inventors and individuals working under their direction (*See, e.g.,* Experimental Reports or Technical Documents having an ER[###] or TD-[###] identification)) from which HyperBranch may derive or ascertain information responsive to this interrogatory. Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery or as Defendant's invalidity contentions are fully and completely provided, in accordance with the Rules.

SUPPLEMENTAL OBJECTION AND ANSWER TO INTERROGATORY NO. 1[9]:

Subject to and without waiving any of its objections, based on information currently available to Plaintiffs and further to the Court's Order during the telephone conference on December 1, 2016, Plaintiffs supplement their previous response by stating that to the extent that Plaintiffs understand this interrogatory, Plaintiffs identify the following individuals who Plaintiffs currently contend to have contributed to the conception of the inventions set forth in the Asserted Claims and Plaintiffs contentions as to the date of conception of the inventions set forth in the Asserted Claims (to the extent that the "Earlier Conception Date" column is blank for any respective row, in the following tables, Plaintiffs are currently relying on the "Earlier Effective Filing Date" as also the "Earlier Conception Date"):

U.S. Patent 7,009,034

Claim	Earlier Effective Filing Dates	Earlier Conception Date*	Inventors
1	December 4, 1998 and December 3, 1999		Pathak
3	December 4, 1998 and December 3, 1999		Pathak
4	December 4, 1998 and December 3, 1999		Pathak
5	December 4, 1998 and December 3, 1999		Pathak
6	November 9, 2001	February 2001	Pathak, Sawhney, Edelman
9	December 4, 1998 and December 3, 1999		Pathak
10	November 9, 2001		Pathak, Sawhney, Edelman
11	December 4, 1998 and December 3, 1999		Pathak
12	December 4, 1998 and December 3, 1999		Pathak
13	December 4, 1998 and December 3, 1999		Pathak
14	December 3, 1999		Pathak
15	December 4, 1998 and December 3, 1999		Pathak
16	December 4, 1998 and December 3, 1999		Pathak
17	December 3, 1999		Pathak
18	December 4, 1998 and December 3, 1999		Pathak
19	December 4, 1998 and December 3, 1999		Pathak
20	December 4, 1998 and December 3, 1999		Pathak
21	December 4, 1998 and December 3, 1999		Pathak

U.S. Patent No. 7,332,566

Claim	Earlier Effective Filing Dates	Earlier Conception Date	Inventors
1	December 4, 1998 and December 3, 1999		Pathak
3	November 9, 2001	February 2001	Pathak, Sawhney, Edelman

4	December 4, 1998 and December 3, 1999		Pathak
6	December 4, 1998 and December 3, 1999		Pathak
7	November 9, 2001		Pathak, Sawhney, Edelman
8	December 4, 1998 and December 3, 1999		Pathak
9	November 9, 2001		Pathak, Sawhney, Edelman
10	December 3, 1999		Pathak
11	December 4, 1998 and December 3, 1999		Pathak
12	December 4, 1998 and December 3, 1999		Pathak
14	December 4, 1998 and December 3, 1999		Pathak
15	November 9, 2001		Pathak, Sawhney, Edelman
16	December 4, 1998 and December 3, 1999		Pathak
18	December 4, 1998 and December 3, 1999		Pathak
19	November 9, 2001		Pathak, Sawhney, Edelman
20	December 4, 1998 and December 3, 1999		Pathak
21	December 4, 1998 and December 3, 1999		Pathak
22	December 4, 1998 and December 3, 1999		Pathak
23	November 9, 2001		Pathak, Sawhney, Edelman
24	December 4, 1998 and December 3, 1999		Pathak
25	December 4, 1998 and December 3, 1999		Pathak
27	November 9, 2001	February 2001	Pathak, Sawhney, Edelman
28	December 4, 1998 and December 3, 1999		Pathak
30	December 4, 1998 and December 3, 1999		Pathak
31	November 9, 2001		Pathak, Sawhney, Edelman
32	December 3, 1999		Pathak

33	December 4, 1998 and December 3, 1999		Pathak
34	November 9, 2001		Pathak, Sawhney, Edelman
35	December 4, 1998 and December 3, 1999		Pathak
36	December 4, 1998 and December 3, 1999		Pathak
37	December 4, 1998 and December 3, 1999		Pathak
38	November 9, 2001		Pathak, Sawhney, Edelman

U.S. Patent No. 7,592,418

Claim	Earlier Effective Filing Dates	Conception Date	Inventors
1	December 4, 1998 and December 3, 1999		Pathak
3	December 4, 1998 and December 3, 1999		Pathak
4	November 9, 2001	February 2001	Pathak, Sawhney, Edelman
5	December 4, 1998 and December 3, 1999		Pathak
6	December 4, 1998 and December 3, 1999		Pathak
7	November 9, 2001		Pathak, Sawhney, Edelman
8	December 3, 1999		Pathak
9	December 4, 1998 and December 3, 1999		Pathak
10	November 9, 2001		Pathak, Sawhney, Edelman
11	December 4, 1998 and December 3, 1999		Pathak
13	December 4, 1998 and December 3, 1999		Pathak
14	December 4, 1998 and December 3, 1999		Pathak
15	December 4, 1998 and December 3, 1999		Pathak
16	December 4, 1998 and December 3, 1999		Pathak
22	December 4, 1998 and December 3, 1999		Pathak

23	December 4, 1998 and December 3, 1999		Pathak
24	December 4, 1998 and December 3, 1999		Pathak
25	December 4, 1998 and December 3, 1999		Pathak
26	November 9, 2001		Pathak, Sawhney, Edelman
27	December 4, 1998 and December 3, 1999		Pathak
28	December 4, 1998 and December 3, 1999		Pathak
29	December 4, 1998 and December 3, 1999		Pathak
30	November 9, 2001		Pathak, Sawhney, Edelman

U.S. Patent No. 6,566,406

Claim	Earlier Effective Filing Dates	Conception Dates	Inventors
1	December 4, 1998		Pathak
2	December 4, 1998		Pathak
6	December 4, 1998		Pathak
7	December 4, 1998		Pathak
8	December 4, 1998		Pathak
10	December 4, 1998		Pathak
12	December 4, 1998		Pathak
14	December 3, 1999		Pathak, Sawhney, Edelman
15	December 3, 1999		Pathak, Sawhney, Edelman
16	December 4, 1998		Pathak
19	December 4, 1998		Pathak
21	December 4, 1998		Pathak
23	December 3, 1999		Pathak, Sawhney, Edelman
24	December 3, 1999		Pathak, Sawhney, Edelman
25	December 3, 1999		Pathak, Sawhney, Edelman

U.S. Patent No. 8,003,705

Claim	Earlier Effective Filing Dates	Conception Dates	Inventors
1	May 28, 2008	December 2000	Sawhney, Bennett, Edelman
4	November 9, 2001		Sawhney, Edelman
5	November 9, 2001		Sawhney, Edelman
6	November 9, 2001		Sawhney, Edelman
11	November 9, 2001		Sawhney, Edelman
12	November 9, 2001		Sawhney, Edelman
13	November 9, 2001		Sawhney, Edelman
16	May 28, 2008	December 2000	Sawhney, Bennett, Edelman
19	May 28, 2008	December 2000	Sawhney, Bennett, Edelman

U.S. Patent No. 8,535,705

Claim	Earlier Effective Filing Dates	Conception Dates	Inventors
1	December 4, 1998 and December 3, 1999		Pathak
5	December 4, 1998 and December 3, 1999		Pathak
6	December 4, 1998 and December 3, 1999		Pathak
7	December 4, 1998 and December 3, 1999		Pathak
9	December 3, 1999		Pathak, Sawhney, Edelman
12	December 4, 1998 and December 3, 1999		Pathak
15	December 4, 1998 and December 3, 1999		Pathak
17	December 4, 1998 and December 3, 1999		Pathak

Plaintiffs reserve the right to amend or supplement this response as this case proceeds.

INTERROGATORY NO. 2 [10]. On a claim-by-claim basis for each and every claim of the Asserted Patents, identify what You contend to be the effective filing date for the claim, including all supporting facts and evidence for the identified effective filing date such as, without limitation, the specific page and lines of any prior filed applications that you contend supports Your identified effective filing date for each claim.

OBJECTION AND ANSWER TO INTERROGATORY NO. 2 [10]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this interrogatory to the extent it purports to be HyperBranch's second interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's tenth interrogatory. Plaintiffs further object to this interrogatory as being unreasonably cumulative or duplicative, or already known to HyperBranch. *See* Plaintiffs' Responses and Supplemental Responses to Interrogatory Nos. 1 and 8 and Rebuttal Expert Report of Dr. Jimmy Mays, hereby incorporated by reference in their entirety. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome and premature at this stage of the litigation in that it requests identification of "all of the factual and legal bases for that contention, and identify all documents and evidence you claim supports that contention." ." Plaintiffs further object to this interrogatory as premature and irrelevant to the extent it is a contention interrogatory that seeks to impose a burden on Plaintiffs to provide a rebuttal position on the effective filing date of each claim prior to the disclosure of any invalidity contention by the Defendant that puts at issue the effective filing date of any claim on which Defendant has the burden of proof and is required to provide its full and complete invalidity contentions. Validity of the claims is presumed by the issuance of the patent. Defendant bears the burden of establishing through its invalidity contentions that there is an issue

as to validity that would require Plaintiffs to prove an earlier effective filing date. To date, Defendants validity contentions have not met that burden. Plaintiffs further object to this Interrogatory to the extent it contains subparts which, together with the other Interrogatories, exceed the limit under the Federal Rules. Plaintiffs also object to this interrogatory to the extent it calls for legal argument and/or expert testimony, which Plaintiffs may provide, in due course and in accordance with the Court's Scheduling Order.

Subject to and without waiving its objections, Plaintiffs rely on the disclosures provided in the patents-in-suit including the related U.S. applications provided on the front of each of the patents in suit to provide an effective filing date for each of the claims. Particularly, the related U.S. applications listed on the face of the patents-in-suit show that the effective filing date for many of the limitations found in the claims of the patents-in-suit may extend back to at least as early as December 4, 1998 and possibly as early as September 23, 1996. For example, many of the limitations claimed in the patents-in-suit can expressly be found in the text of the related U.S. applications. (*See, e.g.*, visualization agent, precursors, biodegradable polymers, biodegradable polymeric crosslinkers, nucleophilic functional groups, electrophilic functional groups, hydrogel film thickness, and many others). Plaintiffs further respond that they have produced non-privileged documents pursuant to Federal Rule of Civil Procedure 33(d) for which the burden of deriving or ascertaining the answer will be substantially the same for HyperBranch as it is for plaintiffs, namely the patents-in-suit, the patents and applications from which the patents-in-suit claim priority, and prosecution histories of these patents and patent applications.

Plaintiffs also identify Exhibits 57 and 58 to the previous deposition of the inventors along with the transcripts of those depositions (i.e., Amar Sawhney and Steven Bennett) as providing

further information related to the effective filing date of the claims of the patents-in-suit. *See, e.g.,* Steve Bennett deposition transcript at pp. 147-48.

Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery and as rebuttal if Defendant meets its burden of setting forth a preliminary contention of invalidity that puts at issue the effective filing date of one or more claims of the patents-in-suit in accordance with the rules and the Scheduling Order in this matter.

SUPPLEMENTAL OBJECTION AND ANSWER TO INTERROGATORY NO. 2[10]:

Subject to and without waiving any of its objections, based on information currently available to Plaintiffs, Plaintiffs supplement their previous response by stating that to the extent that Plaintiffs understand this interrogatory, Plaintiffs incorporate by reference their response to Interrogatory No. 1[9] and all supplements thereto as identifying Plaintiffs current contentions as to the effective filing dates earlier than the filing date of the application that directly issued as the U.S. Patent and supporting evidence for the inventions set forth in the Asserted Claims. Plaintiffs reserve the right to amend or supplement this response as this case proceeds.

AS TO OBJECTIONS ONLY:

DATED: December 9, 2016

/s/ Karen L. Pascale

An Attorney for Plaintiffs, Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical, Inc., and Incept LLC

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(Continued . . . .)

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CERTIFICATE OF SERVICE

I, Karen L. Pascale, Esquire, hereby certify that on December 9, 2016, I caused true and correct copies of the foregoing document to be served upon the following counsel of record by e-mail:

For Defendant HyperBranch Medical Technology, Inc.:

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Attorneys for Plaintiffs Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical Inc., and Incept LLC

Exhibit 3

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

INTEGRA LIFESCIENCES CORP., INTEGRA LIFESCIENCES SALES LLC, CONFLUENT SURGICAL, INC., and INCEPT LLC,	)	
Plaintiffs,	)	
v.	)	C.A. No. 15-819 (LPS) (CJB)
HYPERBRANCH MEDICAL TECHNOLOGY, INC.,	)	
Defendant.	)	

HYPERBRANCH'S PRELIMINARY ELECTION OF ASSERTED PRIOR ART

Pursuant to the Scheduling Order (D.I. 173), as amended by the Court's Oral Order of October 28, 2016 and the parties' stipulation of December 21, 2016 (D.I. 217), Defendant HyperBranch Medical Technology, Inc. ("HyperBranch") identifies the following preliminary invalidity grounds with respect to the thirty-six claims elected on December 23, 2016 by Plaintiffs Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical, Inc. (collectively "Integra"), and Incept LLC (collectively with Integra, "Plaintiffs").

HyperBranch alleges that the claims of U.S. Patent Nos. 7,009,034 (the "'034 patent"), 7,592,418 (the "'418 patent"), 7,332,566 (the "'566 patent"), 6,566,406 (the "'406 patent"), 8,003,705 (the "'3,705 patent"), and 8,535,705 (the "'5,705 patent") (collectively, "the Patents-in-Suit") elected by Plaintiffs (the "Elected Claims") are invalidated by at least the references and/or combinations of references identified below. HyperBranch bases these invalidity grounds upon its current knowledge, understanding, and belief as to the facts and information available as of this date. HyperBranch's investigation is ongoing. Claim construction has not been completed, fact and expert depositions remain to be taken, and Plaintiffs' production of

documents and materials, as well as Plaintiffs' responses to interrogatories and requests for admission, are incomplete and deficient. Further review of discovery produced by Plaintiffs, review of documents produced by any third party, deposition testimony, the investigation and analysis of any testifying expert, or the results of any future investigation may require HyperBranch to supplement these grounds. HyperBranch reserves the right to further supplement, revise, or modify these grounds without prejudice.

In their interrogatory answers dated December 9, 2016, Plaintiffs identified effective filing dates that were prior to the presumptive priority dates for certain Elected Claims. HyperBranch disputes these alleged priority dates. The pool of available prior art depends on the operative priority dates of the Elected Claims. As a result, if Plaintiffs are unable to meet their burden of establishing entitlement to an alleged priority date, additional references after that alleged date would qualify as prior art. Although some of the grounds identified below assume that Plaintiffs are not entitled to certain alleged priority dates, the numerical limit on HyperBranch's preliminary election of invalidity grounds prevents HyperBranch from identifying grounds that sufficiently account for the various priority dates to which Plaintiffs' Elected Claims may or may not be entitled. Should Plaintiffs amend an alleged priority date to a later date for one or more Elected Claims, or should the Court find that Plaintiffs are not entitled to an alleged priority date for one or more Elected Claims, HyperBranch reserves the right to further supplement, revise, or modify these grounds without prejudice.[1]

Furthermore, Plaintiffs have not provided any evidence in support of the alleged priority and conception dates identified in their interrogatory answers dated December 9, 2016, and their responses remain deficient at this time. Accordingly, since it is Plaintiffs' burden to establish a

[1] Under no circumstances should Plaintiffs be allowed to amend an alleged priority date to an earlier date.

date of priority and/or conception that is earlier than the presumptive filing date, HyperBranch further reserves the right to supplement, revise, or modify these grounds without prejudice if Plaintiffs provide any additional information responsive to these interrogatories.

The proper construction of the Elected Claims may impact the relevance of prior art references. The numerical limit on HyperBranch's preliminary election of invalidity grounds likewise prevents HyperBranch from identifying grounds that sufficiently account for the various disputed constructions of claim terms. HyperBranch reserves the right to further supplement, revise, or modify these grounds without prejudice following claim construction.

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
1.	U.S. 5,874,500 (Rhee) (and other related patents or applications with the same or similar disclosures)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 16, 21, 22, 23, 24, 31, 35
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
2.	U.S. 5,614,587 (Rhee) (and other related patents or applications with the same or similar disclosures)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 16, 21, 22, 23, 24, 31, 35
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
3.	U.S. 5,752,974 (Rhee) (and other related patents or applications with the same or similar disclosures)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 16, 21, 22, 23, 24, 31, 35

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
4.	U.S. 5,936,035 (Rhee) (and other related patents or applications with the same or similar disclosures)	7,009,034	4, 5, 10
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
5.	U.S. 5,744,545 (Rhee) (and other related patents or applications with the same or similar disclosures)	7,009,034	4, 5, 10
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
6.	U.S. 5,786,421 (Rhee) (and other related patents or applications with the same or similar disclosures)	7,009,034	4, 5, 10
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
7.	U.S. 5,324,775 (Rhee) (and other related patents or applications with the same or similar disclosures)	6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
8.	U.S. 6,312,725 (Wallace) (and other related patents or applications with the same or similar disclosures)	8,535,705	1, 6, 7, 12, 15, 17
9.	Prestwich, JACS 1994, 7515-7522	6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 17
10.	Gayet, J.C.R. 1996 (177-184)	7,009,034	4, 5, 10
		7,592,418	1, 8, 11, 22, 23, 26
		7,332,566	1, 4, 14, 15, 16, 22, 23, 24, 35
11.	U.S. 5,658,592 (Tanihara) (and other related patents or applications with the same or similar disclosures)	7,009,034	5
		6,566,406	23

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
12.	Prestwich, JACS 1994, 7515-7522 in combination with WO 97/22371 (Rhee)	8,535,705	1, 6, 7, 12, 15, 17
		6,566,406	8, 14, 23
13.	Prestwich, JACS 1994, 7515-7522 in combination with U.S. 5,583,114 (Barrows)	8,535,705	1, 6, 7, 12, 15, 17
		6,566,406	8, 14, 23
14.	WO 97/22371 (Rhee)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 16, 21, 22, 23, 24, 31, 35
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
15.	U.S. 5,583,114 (Barrows) in combination with U.S. 5,874,500 (Rhee)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 16, 21, 22, 23, 24, 31, 35
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
16.	Tse, Arch. Opth. in combination with U.S. 5,874,500 (Rhee)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
17.	U.S. 5,614,587 (Rhee) in combination with WO 98/35631 (Pathak)	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
18.	U.S. 5,874,500 (Rhee) in combination with WO 98/35631 (Pathak)	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
19.	WO 2000/033764 (Pathak) in combination with WO 98/35631 (Pathak)	7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,009,034	4, 5, 10, 20
20.	Tse, Arch. Opth. in combination with U.S. 5,614,587 (Rhee) and U.S. 5,292,362 (Bass)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
21.	Tse, Arch. Opth. in combination with WO 2000/033764 (Pathak)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
22.	U.S. 6,624,245 (Wallace) in combination with U.S. 5,874,500 (Rhee)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
23.	U.S. 6,624,245 (Wallace) in combination with WO 2000/033764 (Pathak)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
24.	U.S. 2008/0114092 (Sawhney) in combination with U.S. 6,258,351 (Harris)	8,003,705	4, 6, 13, 19
25.	U.S. 5,874,500 (Rhee) in combination with U.S. 2008/0114092 (Sawhney)	8,003,705	4, 6, 13, 19
26.	WO 2000/033764 (Pathak) in combination with U.S. 2008/0114092 (Sawhney)	8,003,705	4, 6, 13, 19

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
27.	U.S. 6,165,201 (Sawhney) in combination with U.S. 2008/0114092 (Sawhney)	8,003,705	4, 6, 13, 19
28.	U.S. 7,964,217 (Harris) in combination with WO 98/35631 (Pathak)	7,592,418	1, 7, 8, 11, 22, 23, 26
		6,566,406	8, 14, 23
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		8,535,705	1, 6, 7, 12, 15, 17
		7,009,034	4, 5, 10, 20
29.	U.S. 7,964,217 (Harris) in combination with WO 98/35631 (Pathak) and Tse Arch. Opth.	7,592,418	1, 7, 8, 11, 22, 23, 26
		6,566,406	8, 14, 23
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		8,535,705	1, 6, 7, 12, 15, 17
		7,009,034	4, 5, 10, 20
30.	U.S. 6,258,351 (Harris) in combination with Pathak (WO 98/35631)	7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		8,535,705	1, 6, 7, 12, 15, 17
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
		6,566,406	8, 14, 23
31.	WO 2000/09087 (Sawhney) in combination with U.S. 6,258,351 (Harris)	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
32.	WO 2000/09087 (Sawhney) in combination with U.S. 6,258,351 (Harris) and Tse Arch. Opth.	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
33.	WO 2000/09087 (Sawhney) in combination with	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
	Tse Arch. Opth.	7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
34.	WO 2000/09087 (Sawhney) in combination with 5,874,500 (Rhee) and Tse Arch. Opth.	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
35.	WO 2000/09087 (Sawhney) in combination with U.S. 6,258,351 (Harris) and Pathak (WO 00/33764)	8,535,705	1, 6, 7, 12, 15, 17
		8,003,705	4, 6, 13, 19
		6,566,406	8, 14, 23
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
36.	Rhee 5,874,500 (Rhee) in combination with U.S. 6,258,351 (Harris)	8,535,705	1, 6, 7, 12, 15, 17
		8,003,705	4, 6, 13, 19
		6,566,406	8, 14, 23
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
37.	5,874,500 (Rhee) in combination with U.S. 6,258,351 (Harris) and Tse, Arch. Opth.	8,535,705	1, 6, 7, 12, 15, 17
		8,003,705	4, 6, 13, 19

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
		6,566,406	8, 14, 23
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
38.	5,874,500 (Rhee) in combination with Davis, Emergency Nurse	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
39.	5,874,500 (Rhee) in combination with Davis, Emergency Nurse and U.S. 6,258,351 (Harris)	8,535,705	1, 6, 7, 12, 15, 17
		8,003,705	4, 6, 13, 19
		6,566,406	8, 14, 23
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
40.	5,874,500 (Rhee) in combination with U.S. 5,292,362 (Bass)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		6,566,406	8, 14, 23
		8,535,705	1, 6, 7, 12, 15, 17
41.	Davis, Emergency Nurse in combination with U.S. 5,874,500 (Rhee)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
42.	Davis, Emergency Nurse in combination with	7,009,03	4, 5, 10, 20

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
	U.S. 5,614,587 (Rhee) and U.S. 5,292,362 (Bass)	4	
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
43.	Davis, Emergency Nurse in combination with WO 2000/033764 (Pathak)	7,009,034	4, 5, 10, 20
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
44.	U.S. 7,964,217 (Harris) in combination with WO 98/35631 (Pathak) and Davis, Emergency Nurse.	7,592,418	1, 7, 8, 11, 22, 23, 26
		7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		8,535,705	1, 6, 7, 12, 15, 17
		7,009,034	4, 5, 10, 20
		6,566,406	8, 14, 23
45.	WO 2000/09087 (Sawhney) in combination with U.S. 7,964,217 (Harris) and Davis, Emergency Nurse	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
46.	WO 2000/09087 (Sawhney) in combination with Davis, Emergency Nurse	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19
47.	WO 2000/09087 (Sawhney) in combination with 5,874,500 (Rhee) and Davis, Emergency Nurse	7,332,566	1, 4, 9, 14, 15, 16, 21, 22, 23, 24, 31, 35
		7,592,418	1, 7, 8, 11, 22, 23, 26
		7,009,034	4, 5, 10, 20
		8,003,705	4, 6, 13, 19

Ground Number	References Comprising Anticipation or Obviousness Ground (anticipation grounds are shaded)	Asserted Patent	Claims Rendered Invalid
48.	U.S. 2007/0196454 (Stockman)	8,003,705	4, 6, 13, 19

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January 27, 2017

CERTIFICATE OF SERVICE

I hereby certify that true and correct copies of the foregoing were caused to be served on January 27, 2017 upon the following individuals in the manner indicated:

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Exhibit 4

Exhibit F-14: U.S. Pat. No. 8,535,705 and Wallace 6,312,725

U.S. Pat. No. 8,535,705 and Wallace 6,312,725

Claims 1, 6, 7, 12, 15 and 17 of asserted U.S. Pat. No. 8,535,705 (the "'5,705 patent") are either anticipated or rendered obvious, either alone or in combination with the knowledge of a person of ordinary skill in the art ("POSA"), under 35 U.S.C. §§ 102, 103 by U.S. Pat. No. 6,312,725 ("Wallace '725"), filed on April 16, 1999, and issued on November 6, 2001 (and other related patents or applications with the same or similar disclosures). To the degree that Wallace '725 does not anticipate these claims, the claims would have been obvious to a POSA as further identified in HyperBranch's November 4, 2016 Invalidity Contentions; December 16, 2016 Frist Supplemental Invalidity Contentions; and Second Supplemental Invalidity Contentions.[1]

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
1. A method of making a biocompatible degradable hydrogel to treat a medical condition of a patient comprising: identifying a medical condition for treatment by use of a hydrogel formed in situ in a patient and fully degradable in a patient in less than about 180 days; and	**Wallace '725** In another aspect of the present invention, the compounds further comprise chain extenders between the polymer core and the functional groups. Such chain extenders can activate or suppress reactivity of the functional groups, and can also be used to provide sites for hydrolysis or degradation. Suitable chain extenders include poly(amino acids), poly (lactones), poly(anhydrides), poly(orthoesters), poly (orthocarbonates), poly(phosphoesters), and enzymatically cleavable peptide groups. (2:38-47) Chain Extenders Functional groups may be directly attached to the compound core, or they may be indirectly attached through a chain extender. Such chain extenders are well known in the art. See, for example, PCT WO 97/22371, which describes "linking groups" that would be suitable for use as chain extenders in the compositions of the present invention.

[1] The excerpts cited herein are exemplary. For any claim limitation, Defendant may rely on excerpts cited for any other limitation and/or additional excerpts not set forth fully herein to the extent necessary to provide a more fulsome explanation for a reference's disclosure of a limitation. Where an excerpt refers to or discusses a figure or figure items, that figure and any additional descriptions of that figure should be understood to be incorporated by reference as if set forth fully therein. Where an excerpt contains underlining, bold, or italics, the emphasis is only meant as an example and is not intended to be limiting. The absence of citations to a combination reference for any claim element is not an admission that the combination reference does not teach or fairly suggest that claim element.

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	Chain extenders are useful to avoid stearic hindrance problems that are sometimes associated with the formation of direct linkages between molecules. Alternatively, chain extenders may be used to link several multifunctionally activated compounds together to make larger molecules. In a particularly preferred embodiment, the chain extender can also be used to alter the degradative properties of the compositions after administration and resultant gel formation. For example, chain extenders can be incorporated into one or both of the multifunctionally activated polymers to promote hydrolysis, to discourage hydrolysis, or to provide a site for enzymatic degradation. (7:1-20) Chain extenders may provide sites for degradation, i.e., hydrolysable sites. Examples of hydrolysable chain extend ers include, inter alia, alpha-hydroxy acids such as lactic acid and glycolic acid; poly(lactones) such as caprolactone, valerolactone, gamma butyl lactone and p-dioxanone; poly (amino acids); poly(anhydrides) such as glutarate and suc cinate; poly(orthoesters); poly(orthocarbonates) such as tri methylene carbonate; and poly(phosphoesters). Examples of non-degradable chain extenders include, inter alia, succinimide, propionic acid and carboxymethylate. See, for example, PCT WO 99/07417. Examples of enzymatically degradable chain extenders include Leu-Gly-Pro-Ala, which is degraded by collagenase; and Gly-Pro-Lys, which is degraded by plasmin. (7:32-44) A particularly preferred composition for hemostatic applications to actively bleeding 30 tissue sites comprises a mixture of maleimidyl and succinimidyl

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when compared to compositions having only maleimidyl PEG or 35 succinimidyl PEG alone. (9:29-36) This invention relates generally to two-part polymer com positions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid adhesion to the tissue and gel formation is desired. In particular, they are useful as tissue sealants, in promoting hemostasis, for drug delivery, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions. (Abstract, 1:5-18) In one aspect of the present invention, at least one and preferably both of the compounds are polymers. The non reactive portion of the polymeric compound is referred to as its "core". Suitable polymer cores are synthetic polymers, polyamino acids, and polysaccharides. In a preferred embodiment, the core is a polyalkylene oxide, and more preferably it is polyethylene glycol. (2:12-18) The compositions of the present invention are applied in liquid or solid form to the site of administration. It is also possible to supply them premixed but inactive, and then activate them at the site of administration. In another aspect of the present invention, there is pro vided a method of treating tissues for the purpose of sealing

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	tissues, preventing adhesions, providing a platform for delivery of biologically active agents, or augmenting tissues, comprising mixing together the two components as described herein at the site of administration to produce the desired medical affect. (2:61-3:4) The present invention relates to two-part polymer com positions that form a matrix when mixed together at the site of administration. Each component of the composition is generally administered separately to the tissue site. Then, within a very short time after being mixed together at the site of administration, the composition forms a gel with suffi cient adhesive and cohesive strength to become anchored in place. (3:25-32) The compositions of the present invention can be used in a variety of different pharmaceutical applications. In general, the compositions described herein can be adapted for use in any tissue engineering application where synthetic gel matrices are currently being utilized. For example, the compositions of the present invention are useful as tissue sealants, in tissue augmentation, in tissue repair, as hemostatic agents, in preventing tissue adhesions, in providing surface modifications, and in drug/cell/gene delivery applications. One of skill in the art could easily determine the 20 appropriate administration protocol to use with any composition having a known gel strength and gelation time based on the principles described herein and well known scientific principles. (10:10-24) Tissue Sealants & Adhesives

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	In a preferred application, the compositions described herein can be used for medical conditions that require a coating or sealing layer to prevent the leakage of gases, liquid or solids. The method entails applying both compo ments to the damaged tissue or organ to seal 1) vascular and or other tissues or organs to stop or minimize the flow of blood; 2) thoracic tissue to stop or minimize the leakage of air; 3) gastrointestinal tract or pancreatic tissue to stop or minimize the leakage of fecal or tissue contents; 4) bladder or ureters to stop or minimize the leakage of urine; 5) dura to stop or minimize the leakage of CSF; and 6) skin or serosal tissue to stop the leakage of serosal fluid. These compositions may also be used to adhere tissues together such as small vessels, nerves or dermal tissue. The material can be used 1) by applying it to the surface of one tissue and then a second tissue may be rapidly pressed against the first tissue or 2) by bringing the tissues in close juxtaposition and then applying the material. Surgical Adhesions A preferred application is a method of reducing the formation of adhesions after a surgical procedure in a patient. The method entails applying the material onto the damaged tissue or organ either by spraying both components together or by applying previously admixed components. The components will react together to form a hydrogel on the tissue surface. The medical procedures include gynecological, abdominal, neurosurgical, cardiac, and orthopedic indications. (10:25-53) Example 1 Preparation of a Two-Component Tissue Sealant

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) Example 2 Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63)

TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	-	+
B	fibrillar collagen	-	+
C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400; gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

(col. 13)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show a mild gross and histological response of fibrillar collagen (Collagen Corporation, Palo Alto, Calif.) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved. Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network. This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus increasing the amount of free amines on the polymeric network. This treatment decreases the

<table>
<tr><th>Claims 1, 6, 7, 12, 15 and 17</th><th>Wallace '725</th></tr>
<tr><td></td><td>biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations. (col. 14)

A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 6
Effect Buffers on Gelation Time.

<table>
<tr><th>Test</th><th>Buffer</th><th>Gel Time (Sec)</th></tr>
<tr><td>A</td><td>P/C Buffer</td><td>8</td></tr>
<tr><td>B</td><td>150 mM phosphate</td><td>35</td></tr>
<tr><td>C</td><td>58 mM phosphate
91 mM sodium chloride</td><td>138</td></tr>
<tr><td>D</td><td>58 mM phosphate
91 mM borate</td><td><19</td></tr>
<tr><td>E</td><td>58 mM phosphate
91 mM AMPSO*</td><td>8</td></tr>
</table>
*(3[1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxypropane-sulfonic acid

As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable (col. 15)</td></tr>
<tr><td>mixing a first precursor with a second precursor in situ in the patient to form the hydro gel for treatment of the</td><td><u>Wallace '725</u>
In a variety of tissue engineering applications, it is desirable to use compositions that can be administered as liquids, but subsequently form hydrogels at the site of administration. Such in situ hydrogel forming compositions are more convenient to use since they can be administered as liquids</td></tr>
</table>

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
medical condition,	from a variety of different devices, and are more adaptable for administration to any site, since they are not preformed. (1:24-30.) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) This invention relates generally to two-part polymer com positions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	adhesion to the tissue and gel formation is desired. In particular, they are useful as tissue sealants, in promoting hemostasis, for drug delivery, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions. (Abstract, 1:5-18) see also, Examples 2, 3.
with the first biocompatible synthetic hydrophilic polymer precursor having a water solubility of at least 1 gram per 100 milliliters and	**Wallace '725** Example 12 Synthesis of "12-arm" PEG Compounds A 12-arm electrophilic PEG compound is formed from 1 mole of 4-arm sulfhydryl PEG, 10,000 mol. wt., and 4 moles of 4-arm SG-PEG, 10,000 mol. wt. The resulting compound is depicted in FIG. 4a. As shown, the compound core is pentaerythritol PEG ether tetra-sulfhydryl and the end functional group is succinimide. As long as the functional groups are reactive with one another to form chemical bonds, the sulfhydryl group, X, can be replaced with other nucleophilic groups, such as NH2, etc., and the succinimidyl group, Y, can be replaced with other electrophilic groups, such as maleimide, carbonyl imidazole, or isocyanate. This method is also used to prepare the 12-arm nucleophilic PEG compound depicted in FIG. 4b by reacting 4 moles of 4-arm sulfhydryl PEG with 1 mole of 4-arm SG-PEG. The formation of these compounds from their respective 4-arm intermediates are also shown in FIG. 5. It should be understood that such reactions produce a heterogeneous population of activated PEG product, some having less than 12 arms, and some having more than 12 arms. As used herein, a "12-arm" PEG also refers to such heterogeneous reaction products that have an average of about 12 arms on each molecule. (18:15-19:7) Composition Components The two-part compositions of the present invention com prise two different compounds, each within a separate part of the composition and at least one of which is a polymer, that react with one another to form a covalently crosslinked gel matrix. As such, they can easily be administered separately, and rapidly form gels at the site of administration. In the compositions of the present invention, each com

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	ponent is present in one of the two separate parts, or "components", of the composition, along with other optional ingredients as described elsewhere herein. The two reactive compounds and the gel matrix that forms when they are mixed together can be represented by Formula I as follows: Compound?—(SH),4-Compound2–Ya-> Compoundl–Z-Compound, (I) Compound, has multiple (m=2) sulfhydryl groups (SH) that react with Compounds, which has multiple (n=2) sulfhydryl-reactive groups (Y). It should be understood that sulfhydryl groups are also "sulfhydryl reactive groups", since it is well known that sulfhydryl groups will react with one another under certain conditions. When mixed together, the two compounds become interconnected via a covalent bond (Z). As depicted in FIG. 1 for illustration purposes only, there is only one bond formed between Compound, and Compound2. However, when m--n?5, and appropriate ratios of the two components are utilized as described elsewhere herein, the two compounds form multiple attach ments to one another resulting in a three-dimensional poly mer matrix. Preferably, both compounds contain four or more functional groups, since such multifunctionality results in a gel matrix with greater overall cohesive strength. In a particularly preferred embodiment, each of the compounds is tetrafunctionally activated. In another preferred embodiment, the compounds each 55 have 12 functional groups. Such compounds are formed from reacting a first tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" function-

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	60 ally activated polymers. An example of such a "12-arm" compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size 65 from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials. (4:8-65)
comprising at least two electrophilic functional groups;	**Wallace '725** Example 12 Synthesis of "12-arm" PEG Compounds A 12-arm electrophilic PEG compound is formed from 1 mole of 4-arm sulfhydryl PEG, 10,000 mol. wt., and 4 moles of 4-arm SG-PEG, 10,000 mol. wt. The resulting compound is depicted in FIG. 4a. As shown, the compound core is pentaerythritol PEG ether tetra-sulfhydryl and the end functional group is succinimide. As long as the functional groups are reactive with one another to form chemical bonds, the sulfhydryl group, X, can be replaced with other nucleophilic groups, such as NH2, etc., and the succinimidyl group, Y, can be replaced with other electrophilic groups, such as maleimide, carbonyl imidazole, or isocyanate. This method is also used to prepare the 12-arm nucleophilic PEG compound depicted in FIG. 4b by reacting 4 moles of 4-arm sulfhydryl PEG with 1 mole of 4-arm SG-PEG. The formation of these compounds from their respective 4-arm intermediates are also shown in FIG. 5. It should be understood that such reactions produce a heterogeneous population of activated PEG product, some having less than 12 arms, and some having more than 12 arms. As used herein, a "12-arm" PEG also refers to such heterogeneous reaction products that have an average of about 12 arms on each molecule. (18:15-19:7) Composition Components The two-part compositions of the present invention com prise two different compounds, each within a separate part of the composition and at least one of which is a polymer, that react with one another to form a covalently crosslinked

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	gel matrix. As such, they can easily be administered separately, and rapidly form gels at the site of administration. In the compositions of the present invention, each com ponent is present in one of the two separate parts, or "components", of the composition, along with other optional ingredients as described elsewhere herein. The two reactive compounds and the gel matrix that forms when they are mixed together can be represented by Formula I as follows: Compound?—(SH),4-Compound2–Ya-> Compoundl–Z-Compound, (I) Compound, has multiple (m=2) sulfhydryl groups (SH) that react with Compounds, which has multiple (n=2) sulfhydryl-reactive groups (Y). It should be understood that sulfhydryl groups are also "sulfhydryl reactive groups", since it is well known that sulfhydryl groups will react with one another under certain conditions. When mixed together, the two compounds become interconnected via a covalent bond (Z). As depicted in FIG. 1 for illustration purposes only, there is only one bond formed between Compound, and Compound2. However, when m--n?5, and appropriate ratios of the two components are utilized as described elsewhere herein, the two compounds form multiple attach ments to one another resulting in a three-dimensional poly mer matrix. Preferably, both compounds contain four or more functional groups, since such multifunctionality results in a gel matrix with greater overall cohesive strength. In a particularly preferred embodiment, each of the compounds is tetrafunctionally activated. In another preferred embodiment, the compounds each 55 have 12 functional groups. Such compounds are formed

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	from reacting a first tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" function- 60 ally activated polymers. An example of such a "12-arm" compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size 65 from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials. (4:8-65)
and the second biocompatible synthetic hydrophilic polymer precursor comprising at least two nucleophilic amine functional groups; and wherein	**Wallace '725** Composition Components The two-part compositions of the present invention com prise two different compounds, each within a separate part of the composition and at least one of which is a polymer, that react with one another to form a covalently crosslinked gel matrix. As such, they can easily be administered separately, and rapidly form gels at the site of administration. In the compositions of the present invention, each com ponent is present in one of the two separate parts, or "components", of the composition, along with other optional ingredients as described elsewhere herein. The two reactive compounds and the gel matrix that forms when they are mixed together can be represented by Formula I as follows: Compound?—(SH),4-Compound2–Ya-> Compoundl–Z-Compound, (I) Compound, has multiple (m=2) sulfhydryl groups (SH)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	that react with Compounds, which has multiple (n=2) sulfhydryl-reactive groups (Y). It should be understood that sulfhydryl groups are also "sulfhydryl reactive groups", since it is well known that sulfhydryl groups will react with one another under certain conditions. When mixed together, the two compounds become interconnected via a covalent bond (Z). As depicted in FIG. 1 for illustration purposes only, there is only one bond formed between Compound, and Compound2. However, when m--n?5, and appropriate ratios of the two components are utilized as described elsewhere herein, the two compounds form multiple attach ments to one another resulting in a three-dimensional poly mer matrix. Preferably, both compounds contain four or more functional groups, since such multifunctionality results in a gel matrix with greater overall cohesive strength. In a particularly preferred embodiment, each of the compounds is tetrafunctionally activated. In another preferred embodiment, the compounds each 55 have 12 functional groups. Such compounds are formed from reacting a first tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" function- 60 ally activated polymers. An example of such a "12-arm" compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size 65 from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials. (4:8-65)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
(i) the first precursor is selected have only one or two chemically hydrolytically degradable ester bonds per every electrophilic functional group on the first precursor; and	**Wallace '725** A particularly preferred composition for hemostatic applications to actively bleeding 30 tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when compared to compositions having only maleimidyl PEG or 35 succinimidyl PEG alone. (9:29-36) In another aspect of the present invention, the compounds further comprise chain extenders between the polymer core and the functional groups. Such chain extenders can activate or suppress reactivity of the functional groups, and can also be used to provide sites for hydrolysis or degradation. Suitable chain extenders include poly(amino acids), poly (lactones), poly(anhydrides), poly(orthoesters), poly (orthocarbonates), poly(phosphoesters), and enzymatically cleavable peptide groups. (2:38-47) Chain Extenders Functional groups may be directly attached to the compound core, or they may be indirectly attached through a chain extender. Such chain extenders are well known in the art. See, for example, PCT WO 97/22371, which describes "linking groups" that would be suitable for use as chain extenders in the compositions of the present invention. Chain extenders are useful to avoid stearic hindrance problems that are sometimes associated with the formation of direct linkages between molecules. Alternatively, chain

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	extenders may be used to link several multifunctionally activated compounds together to make larger molecules. In a particularly preferred embodiment, the chain extender can also be used to alter the degradative properties of the compositions after administration and resultant gel formation. For example, chain extenders can be incorporated into one or both of the multifunctionally activated polymers to promote hydrolysis, to discourage hydrolysis, or to provide a site for enzymatic degradation. (7:1-20) Chain extenders may provide sites for degradation, i.e., hydrolysable sites. Examples of hydrolysable chain extend ers include, inter alia, alpha-hydroxy acids such as lactic acid and glycolic acid; poly(lactones) such as caprolactone, valerolactone, gamma butyl lactone and p-dioxanone; poly (amino acids); poly(anhydrides) such as glutarate and suc cinate; poly(orthoesters); poly(orthocarbonates) such as tri methylene carbonate; and poly(phosphoesters). Examples of non-degradable chain extenders include, inter alia, succinimide, propionic acid and carboxymethylate. See, for example, PCT WO 99/07417. Examples of enzymatically degradable chain extenders include Leu-Gly-Pro-Ala, which is degraded by collagenase; and Gly-Pro-Lys, which is degraded by plasmin. (7:32-44) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) Example 2 Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	-	+
B	fibrillar collagen	-	+
C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400); gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

(col. 13)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show a mild gross and histological response of fibrillar collagen (Collagen Corporation, Palo Alto, Calif.) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved. Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network. This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus increasing the amount of free amines on the polymeric network. This treatment decreases the

<table>
<tr><th>Claims 1, 6, 7, 12, 15 and 17</th><th>Wallace '725</th></tr>
<tr><td></td><td>biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations. (col. 14)

A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 6
Effect Buffers on Gelation Time.

<table>
<tr><th>Test</th><th>Buffer</th><th>Gel Time (Sec)</th></tr>
<tr><td>A</td><td>P/C Buffer</td><td>8</td></tr>
<tr><td>B</td><td>150 mM phosphate</td><td>35</td></tr>
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91 mM sodium chloride</td><td>138</td></tr>
<tr><td>D</td><td>58 mM phosphate
91 mM borate</td><td><19</td></tr>
<tr><td>E</td><td>58 mM phosphate
91 mM AMPSO*</td><td>8</td></tr>
</table>
*(3[1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxypropane-sulfonic acid

As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable (col. 15)</td></tr>
<tr><td>(ii) the second precursor comprises at least three nucleophilic functional groups;</td><td><u>Wallace '725</u>

This invention relates generally to two-part polymer com positions that rapidly form covalent linkages when mixed</td></tr>
</table>

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid adhesion to the tissue and gel formation is desired. In particular, they are useful as tissue sealants, in promoting hemostasis, for drug delivery, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions. (Abstract, 1:5-18) In yet another approach, two-part synthetic polymer compositions have been described that, when mixed together, form covalent bonds with one another, as well as with exposed tissue surfaces. (PCTWO 97/22371, which corresponds to U.S. application Ser. No. 08/769,806 U.S. Pat. No. 5,874,500.) In a similar approach involving a two-part composition, a mixture of protein and a bifunctional crosslinking agent has been described for use as a tissue adhesive (U.S. Pat. No. 5,583, 114.) (1:42-51) The present invention discloses generally two-component polymer compositions that, when mixed together, rapidly react to form a matrix at the site of administration. Such compositions exhibit gel times of less than one minute. In one aspect of the present invention, one of the components is a sulfhydryl-containing compound. In another aspect of the present invention, both components contain multiple functional groups, and at least one of the compounds con tains three or more functional groups. This ensures sufficient reactivity for formation of a three-dimensional polymer matrix. Preferably, both compounds contain four or more functional groups. For extremely fast reacting compositions, both compounds contain 12 functional groups. (1:65-2:11)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	The present invention relates to two-part polymer com positions that form a matrix when mixed together at the site of administration. Each component of the composition is generally administered separately to the tissue site. Then, within a very short time after being mixed together at the site of administration, the composition forms a gel with suffi cient adhesive and cohesive strength to become anchored in place. (3:25-32) The term "activated synthetic polymers" refers to syn thetic polymers that have or have been chemically modified to have at least one functional group (e.g., a sulfhydryl group) that is capable of reacting with a corresponding reaction partner (e.g., a sulfhydryl-reactive group) to form a covalent bond. The term "multifunctionally activated" refers to synthetic polymers having two or more nucleophilic or electrophilic groups. Types of multifunctionally activated synthetic polymers include di-functionally activated, tri functionally activated, tetra-functionally activated, and star shaped activated polymers (that have four or more func tional groups). Composition Components The two-part compositions of the present invention com prise two different compounds, each within a separate part of the composition and at least one of which is a polymer, that react with one another to form a covalently crosslinked gel matrix. As such, they can easily be administered separately, and rapidly form gels at the site of administration. In the compositions of the present invention, each com

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	ponent is present in one of the two separate parts, or "components", of the composition, along with other optional ingredients as described elsewhere herein. The two reactive compounds and the gel matrix that forms when they are mixed together can be represented by Formula I as follows: Compound?—(SH),4-Compound2–Ya-> Compoundl–Z-Compound, (I) Compound, has multiple (m=2) sulfhydryl groups (SH) that react with Compounds, which has multiple (n=2) sulfhydryl-reactive groups (Y). It should be understood that sulfhydryl groups are also "sulfhydryl reactive groups", since it is well known that sulfhydryl groups will react with one another under certain conditions. When mixed together, the two compounds become interconnected via a covalent bond (Z). As depicted in FIG. 1 for illustration purposes only, there is only one bond formed between Compound, and Compound2. However, when m--n?5, and appropriate ratios of the two components are utilized as described elsewhere herein, the two compounds form multiple attach ments to one another resulting in a three-dimensional poly mer matrix. Preferably, both compounds contain four or more functional groups, since such multifunctionality results in a gel matrix with greater overall cohesive strength. In a particularly preferred embodiment, each of the compounds is tetrafunctionally activated. In another preferred embodiment, the compounds each 55 have 12 functional groups. Such compounds are formed from reacting a first tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" function-

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	60 ally activated polymers. An example of such a "12-arm" compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size 65 from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials. (4:8-65) Multifunctionally activated polyalkylene oxides, such as polyethylene glycol, are commercially Available, and are also easily prepared using known methods. For example, see Chapter 22 of Poly(ethylene Glycol) Chemistry: Biotechni cal and Biomedical Applications, J. Milton Harris, ed., Plenum Press, NY (1992); and Shearwater Polymers, Inc. Catalog, Polyethylene Glycol Derivatives, Huntsville, Ala. (1997–1998). For use as a tissue sealant, the preferred combination of activated polymers is as follows: the sulfhydry-reactive group-containing compound is the tet rafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra-succinimidylglutarate (10,000 mol, wt.); and the sulfhydryl group-containing compound is the tetrafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra sulfhydryl (10,000 mol, wt.). In both cases, these "four-arm" PEGs are formed by ethoxylation of pentaerythritol, where each of the four chains is approximately 2,500 mol. wi., and then derivatized to introduce the functional groups onto each of the four arms. Also preferred are analogous poly(ethylene glycol)-like compounds polymerized from di-glycerol instead of pentaerythritol. (5:35-55) Reactive Groups and Matrix Linkages

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	In the present invention, the linkage, Z, comprises a covalent bond between the sulfur atom in the sulfhydryl group-containing compound and, e.g., <* the carbon or sulfur atom in the sulfhydryl-reactive group-containing compound. Accordingly, the linkage may be a thioester, a thioether, a disulfide, or the like. A wide variety of sulfhydryl-reactive groups and the types of linkages they form when reacted with sulfhydryl groups are well known in the scientific literature. For example, see Bodanszky, M., Principles of Peptide Synthesis, 2nd ed., pages 21 to 37, Springer-Verlog, Berlin (1993); and Lundbland, R. L., Chemical Reagents for Protein Modification, 2nd ed., Chapter 6, CRC Press, Boca Raton, Fla. (1991). For most applications, sulfhydryl reactive groups that react with sulfhydryl groups to form thioester linkages are preferred. Such compounds are depicted in FIG. 1 and include, inter alia, the following compounds, with the numbers in parentheses corresponding to the structures shown in FIG. 1: mixed anhydrides, such as PEG-glutaryl-acetyl-anhydride (1), PEG-glutaryl-isovaleryl-anhydride (2), PEG-glutaryl-pivalyl-anhydride (3) and related compounds as presented in Bodanszky, p. 23; Ester derivatives of phosphorus, such as structures (4) and (5); ester derivatives of p-nitrophenol (6) of p-nitrothiophenol (7), of pentafluorophenol (8), of structure (9) and related active esters as presented by Bodanszky, pp. 31-32, and Table 2; esters of substituted hydroxylamines, such as those of N-hydroxy-phthalimide (10), N-hydroxy-succinimide (11), and N-hydroxy-glutarimide (12), as well as related structures in Bodanszky; Table 3; esters of 1-hydroxybenzotriazole (13), 3-hydroxy-3,4-dihydro-benzotriazine-4-one (14) and 3-hydroxy-3,4-dihydro-quinazoline-4-one; derivatives of carbonylimidazole; and isocyanates. With these compounds, auxiliary reagents can also be used to facilitate bond formation, such as 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide can be used to facilitate coupling of carboxyl groups (i.e., glutarate and succinate) with sulfhydryl groups. In addition to the sulfhydryl reactive compounds that form thioester linkages, various other compounds can be utilized that form other types of linkages. For example, compounds that contain methyl imidate derivatives form imido-thioester linkages with sulfhydryl groups. Alternatively, sulfhydryl reactive groups can be employed that form disulfide bonds with sulfhydryl groups, such as ortho pyridyl disulfide, 3-nitro-2-pyridenesulfenyl, 2-nitro-5-thiocyanobenzoic acid, 5,5′-dithio-bis(2-nitrobenzoic acid), derivatives of methane-thiosulfate, and 2,4-dinitrophenyl cysteinyl disulfides. In such instances, auxiliary reagents, such as the hydrogen peroxide or

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	di-tert-butyl ester of azodicarboxylic acid, can be used to facilitiate disulfide bond formation. Yet another class of sulfhydryl reactive groups form thioether bonds with sulfhydryl groups. Such groups include, inter alia, iodoacetamide, N-ethylmaleimide and other maleimides, including dextran maleimides, mono-bromo-bimane and related compounds, vinylsulfones, epoxides, derivatives of O-methyl-isourea, ethyleneimines, aziridines, and 4-(aminosulfonyl-)7-fluoro-2,1,3-benzoxadiazole. (6:8-67) Tissue Sealants & Adhesives In a preferred application, the compositions described herein can be used for medical conditions that require a coating or sealing layer to prevent the leakage of gases, liquid or solids. The method entails applying both compo ments to the damaged tissue or organ to seal 1) vascular and or other tissues or organs to stop or minimize the flow of blood; 2) thoracic tissue to stop or minimize the leakage of air; 3) gastrointestinal tract or pancreatic tissue to stop or minimize the leakage of fecal or tissue contents; 4) bladder or ureters to stop or minimize the leakage of urine; 5) dura to stop or minimize the leakage of CSF; and 6) skin or serosal tissue to stop the leakage of serosal fluid. These compositions may also be used to adhere tissues together such as small vessels, nerves or dermal tissue. The material can be used 1) by applying it to the surface of one tissue and then a second tissue may be rapidly pressed against the first tissue or 2) by bringing the tissues in close juxtaposition and then applying the material. Surgical Adhesions A preferred application is a method of reducing the formation of adhesions after a surgical procedure in a patient. The method entails applying the material onto the damaged tissue or organ either by spraying both components

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	together or by applying previously admixed components. The components will react together to form a hydrogel on the tissue surface. The medical procedures include gynecological, abdominal, neurosurgical, cardiac, and orthopedic indications. (10:25-53) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) Example 2

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	-	+
B	fibrillar collagen	-	+
C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400); gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

(col. 13)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show a mild gross and histological response of fibrillar collagen (Collagen Corporation, Palo Alto, Calif.) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved. Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network. This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus increasing the amount of free amines on the polymeric network. This treatment decreases the

<table>
<tr><th>Claims 1, 6, 7, 12, 15 and 17</th><th>Wallace '725</th></tr>
<tr><td></td><td>biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations. (col. 14)

A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 6
Effect Buffers on Gelation Time.

<table>
<tr><th>Test</th><th>Buffer</th><th>Gel Time (Sec)</th></tr>
<tr><td>A</td><td>P/C Buffer</td><td>8</td></tr>
<tr><td>B</td><td>150 mM phosphate</td><td>35</td></tr>
<tr><td>C</td><td>58 mM phosphate
91 mM sodium chloride</td><td>138</td></tr>
<tr><td>D</td><td>58 mM phosphate
91 mM borate</td><td><19</td></tr>
<tr><td>E</td><td>58 mM phosphate
91 mM AMPSO*</td><td>8</td></tr>
</table>
*(3[1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxypropane-sulfonic acid

As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable (col. 15)</td></tr>
<tr><td>wherein the biodegradable groups of the hydrogel consist of the esters and the hydrogel as placed in situ in the patient is essentially fully</td><td><u>Wallace '725</u>
In another aspect of the present invention, the compounds further comprise chain extenders between the polymer core and the functional groups. Such chain extenders can activate or suppress reactivity of the functional groups, and can also</td></tr>
</table>

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
degradable in a patient in less than about 180 days, and	be used to provide sites for hydrolysis or degradation. Suitable chain extenders include poly(amino acids), poly (lactones), poly(anhydrides), poly(orthoesters), poly (orthocarbonates), poly(phosphoesters), and enzymatically cleavable peptide groups. (2:38-47) Chain Extenders Functional groups may be directly attached to the compound core, or they may be indirectly attached through a chain extender. Such chain extenders are well known in the art. See, for example, PCT WO 97/22371, which describes "linking groups" that would be suitable for use as chain extenders in the compositions of the present invention. Chain extenders are useful to avoid stearic hindrance problems that are sometimes associated with the formation of direct linkages between molecules. Alternatively, chain extenders may be used to link several multifunctionally activated compounds together to make larger molecules. In a particularly preferred embodiment, the chain extender can also be used to alter the degradative properties of the compositions after administration and resultant gel formation. For example, chain extenders can be incorporated into one or both of the multifunctionally activated polymers to promote hydrolysis, to discourage hydrolysis, or to provide a site for enzymatic degradation. (7:1-20) Chain extenders may provide sites for degradation, i.e., hydrolysable sites. Examples of hydrolysable chain extend ers include, inter alia, alpha-hydroxy acids such as lactic acid and glycolic acid; poly(lactones) such as caprolactone, valerolactone, gamma butyl lactone and p-dioxanone; poly

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	(amino acids); poly(anhydrides) such as glutarate and succinate; poly(orthoesters); poly(orthocarbonates) such as trimethylene carbonate; and poly(phosphoesters). Examples of non-degradable chain extenders include, inter alia, succinimide, propionic acid and carboxymethylate. See, for example, PCT WO 99/07417. Examples of enzymatically degradable chain extenders include Leu-Gly-Pro-Ala, which is degraded by collagenase; and Gly-Pro-Lys, which is degraded by plasmin. (7:32-44) A particularly preferred composition for hemostatic applications to actively bleeding tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when compared to compositions having only maleimidyl PEG or succinimidyl PEG alone. (9:29-36) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concentration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydrolysis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) Example 2 Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	-	+
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C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400); gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

(col. 13)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
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B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show a mild gross and histological response of fibrillar collagen (Collagen Corporation, Palo Alto, Calif.) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved. Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network. This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus increasing the amount of free amines on the polymeric network. This treatment decreases the

<table>
<tr><th>Claims 1, 6, 7, 12, 15 and 17</th><th>Wallace '725</th></tr>
<tr><td></td><td>biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations. (col. 14)

A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 6
Effect Buffers on Gelation Time.

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91 mM AMPSO*</td><td>8</td></tr>
</table>
*(3[1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxypropane-sulfonic acid

As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable (col. 15)</td></tr>
<tr><td>wherein mixing the first and the second synthetic hydrophilic polymer precursors forms</td><td><u>Wallace '725</u>
This invention relates generally to two-part polymer com positions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for</td></tr>
</table>

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
crosslinking covalent bonds that are reaction products of the electrophilic and the nucleophilic groups,	use in a variety of tissue related applications when rapid adhesion to the tissue and gel formation is desired. In particular, they are useful as tissue sealants, in promoting hemostasis, for drug delivery, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions. (Abstract, 1:5-18) In yet another approach, two-part synthetic polymer compositions have been described that, when mixed together, form covalent bonds with one another, as well as with exposed tissue surfaces. (PCTWO 97/22371, which corresponds to U.S. application Ser. No. 08/769,806 U.S. Pat. No. 5,874,500.) In a similar approach involving a two-part composition, a mixture of protein and a bifunctional crosslinking agent has been described for use as a tissue adhesive (U.S. Pat. No. 5,583, 114.) (1:42-51) The term "activated synthetic polymers" refers to syn thetic polymers that have or have been chemically modified to have at least one functional group (e.g., a sulfhydryl group) that is capable of reacting with a corresponding reaction partner (e.g., a sulfhydryl-reactive group) to form a covalent bond. The term "multifunctionally activated" refers to synthetic polymers having two or more nucleophilic or electrophilic groups. Types of multifunctionally activated synthetic polymers include di-functionally activated, tri functionally activated, tetra-functionally activated, and star shaped activated polymers (that have four or more func tional groups). Composition Components

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	The two-part compositions of the present invention com prise two different compounds, each within a separate part of the composition and at least one of which is a polymer, that react with one another to form a covalently crosslinked gel matrix. As such, they can easily be administered separately, and rapidly form gels at the site of administration. In the compositions of the present invention, each com ponent is present in one of the two separate parts, or "components", of the composition, along with other optional ingredients as described elsewhere herein. The two reactive compounds and the gel matrix that forms when they are mixed together can be represented by Formula I as follows: Compound?—(SH),4-Compound2–Ya-> Compoundl–Z-Compound, (I) Compound, has multiple (m=2) sulfhydryl groups (SH) that react with Compounds, which has multiple (n=2) sulfhydryl-reactive groups (Y). It should be understood that sulfhydryl groups are also "sulfhydryl reactive groups", since it is well known that sulfhydryl groups will react with one another under certain conditions. When mixed together, the two compounds become interconnected via a covalent bond (Z). As depicted in FIG. 1 for illustration purposes only, there is only one bond formed between Compound, and Compound2. However, when m--n?5, and appropriate ratios of the two components are utilized as described elsewhere herein, the two compounds form multiple attach ments to one another resulting in a three-dimensional poly mer matrix. Preferably, both compounds contain four or more functional groups, since such multifunctionality results in a gel matrix with greater overall cohesive strength. In a particularly preferred embodiment, each of the compounds

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	is tetrafunctionally activated. In the present invention, the linkage, Z, comprises a covalent bond between the sulfur atom in the sulfhydryl group-containing compound and, e.g., <* the carbon or sulfur atom in the sulfhydryl-reactive group-containing compound. Accordingly, the linkage may be a thioester, a thioether, a disulfide, or the like. A wide variety of sulfhydryl-reactive groups and the types of linkages they form when reacted with sulfhydryl groups are well known in the scientific literature. For example, see Bodanszky, M., Principles of Peptide Synthesis, 2nd ed., pages 21 to 37, Springer-Verlog, Berlin (1993); and Lundbland, R. L., Chemical Reagents for Protein Modification, 2nd ed., Chapter 6, CRC Press, Boca Raton, Fla. (1991).
wherein essentially every ester bond in the hydrogel is separated from other ester bonds in the hydrogel by at least three covalent bonds when the hydrogel is formed.	**Wallace '725** In another aspect of the present invention, the compounds further comprise chain extenders between the polymer core and the functional groups. Such chain extenders can activate or suppress reactivity of the functional groups, and can also be used to provide sites for hydrolysis or degradation. Suitable chain extenders include poly(amino acids), poly (lactones), poly(anhydrides), poly(orthoesters), poly (orthocarbonates), poly(phosphoesters), and enzymatically cleavable peptide groups. (2:38-47) Chain Extenders Functional groups may be directly attached to the compound core, or they may be indirectly attached through a chain extender. Such chain extenders are well known in the art. See, for example, PCT WO 97/22371, which describes "linking groups" that would be suitable for use as chain extenders in the compositions of the present invention. Chain extenders are useful to avoid stearic hindrance problems that are sometimes associated with the formation of direct linkages between molecules. Alternatively, chain

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	extenders may be used to link several multifunctionally activated compounds together to make larger molecules. In a particularly preferred embodiment, the chain extender can also be used to alter the degradative properties of the compositions after administration and resultant gel formation. For example, chain extenders can be incorporated into one or both of the multifunctionally activated polymers to promote hydrolysis, to discourage hydrolysis, or to provide a site for enzymatic degradation. (7:1-20) Chain extenders may provide sites for degradation, i.e., hydrolysable sites. Examples of hydrolysable chain extend ers include, inter alia, alpha-hydroxy acids such as lactic acid and glycolic acid; poly(lactones) such as caprolactone, valerolactone, gamma butyl lactone and p-dioxanone; poly (amino acids); poly(anhydrides) such as glutarate and suc cinate; poly(orthoesters); poly(orthocarbonates) such as tri methylene carbonate; and poly(phosphoesters). Examples of non-degradable chain extenders include, inter alia, succinimide, propionic acid and carboxymethylate. See, for example, PCT WO 99/07417. Examples of enzymatically degradable chain extenders include Leu-Gly-Pro-Ala, which is degraded by collagenase; and Gly-Pro-Lys, which is degraded by plasmin. (7:32-44) A particularly preferred composition for hemostatic applications to actively bleeding 30 tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	compared to compositions having only maleimidyl PEG or 35 succinimidyl PEG alone. (9:29-36) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) Example 2 Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63)

TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	-	+
B	fibrillar collagen	-	+
C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400; gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

(col. 13)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show a mild gross and histological response of fibrillar collagen (Collagen Corporation, Palo Alto, Calif.) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved. Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network. This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus

<table>
<tr><th>Claims 1, 6, 7, 12, 15 and 17</th><th>Wallace '725</th></tr>
<tr><td></td><td>increasing the amount of free amines on the polymeric network. This treatment decreases the biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations. (col. 14)

A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 6
Effect Buffers on Gelation Time.

<table>
<tr><th>Test</th><th>Buffer</th><th>Gel Time (Sec)</th></tr>
<tr><td>A</td><td>P/C Buffer</td><td>8</td></tr>
<tr><td>B</td><td>150 mM phosphate</td><td>35</td></tr>
<tr><td>C</td><td>58 mM phosphate
91 mM sodium chloride</td><td>138</td></tr>
<tr><td>D</td><td>58 mM phosphate
91 mM borate</td><td><19</td></tr>
<tr><td>E</td><td>58 mM phosphate
91 mM AMPSO*</td><td>8</td></tr>
</table>
*(3[1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxypropane-sulfonic acid

As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable (col. 15)</td></tr>
<tr><td>6. The method of claim 1 wherein the medical condition is tissue</td><td><u>Wallace '725</u>
In another aspect of the present invention, there is pro
vided a method of treating tissues for the purpose of sealing</td></tr>
</table>

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
sealing.	tissues, preventing adhesions, providing a platform for delivery of biologically active agents, or augmenting tissues, comprising mixing together the two components as described herein at the site of administration to produce the desired medical affect. (2:61-3:4) Tissue Sealants & Adhesives In a preferred application, the compositions described herein can be used for medical conditions that require a coating or sealing layer to prevent the leakage of gases, liquid or solids. The method entails applying both compo ments to the damaged tissue or organ to seal 1) vascular and or other tissues or organs to stop or minimize the flow of blood; 2) thoracic tissue to stop or minimize the leakage of air; 3) gastrointestinal tract or pancreatic tissue to stop or minimize the leakage of fecal or tissue contents; 4) bladder or ureters to stop or minimize the leakage of urine; 5) dura to stop or minimize the leakage of CSF; and 6) skin or serosal tissue to stop the leakage of serosal fluid. These compositions may also be used to adhere tissues together such as small vessels, nerves or dermal tissue. The material can be used 1) by applying it to the surface of one tissue and then a second tissue may be rapidly pressed against the first tissue or 2) by bringing the tissues in close juxtaposition and then applying the material. Surgical Adhesions A preferred application is a method of reducing the formation of adhesions after a surgical procedure in a patient. The method entails applying the material onto the damaged tissue or organ either by spraying both components together or by applying previously admixed components.

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	The components will react together to form a hydrogel on the tissue surface. The medical procedures include gynecological, abdominal, neurosurgical, cardiac, and orthopedic indications. (10:25-53) Example 2 Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63) This invention relates generally to two-part polymer com positions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid adhesion to the tissue and gel formation is desired. In particular, they are useful as tissue sealants, in promoting hemostasis, for drug delivery, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions. (Abstract, 1:5-18)
7. The method of claim 1 wherein the medical condition is tissue coating.	**Wallace '725** Tissue Sealants & Adhesives In a preferred application, the compositions described herein can be used for medical conditions that require a

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	coating or sealing layer to prevent the leakage of gases, liquid or solids. The method entails applying both compo ments to the damaged tissue or organ to seal 1) vascular and or other tissues or organs to stop or minimize the flow of blood; 2) thoracic tissue to stop or minimize the leakage of air; 3) gastrointestinal tract or pancreatic tissue to stop or minimize the leakage of fecal or tissue contents; 4) bladder or ureters to stop or minimize the leakage of urine; 5) dura to stop or minimize the leakage of CSF; and 6) skin or serosal tissue to stop the leakage of serosal fluid. These compositions may also be used to adhere tissues together such as small vessels, nerves or dermal tissue. The material can be used 1) by applying it to the surface of one tissue and then a second tissue may be rapidly pressed against the first tissue or 2) by bringing the tissues in close juxtaposition and then applying the material. Surgical Adhesions A preferred application is a method of reducing the formation of adhesions after a surgical procedure in a patient. The method entails applying the material onto the damaged tissue or organ either by spraying both components together or by applying previously admixed components. The components will react together to form a hydrogel on the tissue surface. The medical procedures include gynecological, abdominal, neurosurgical, cardiac, and orthopedic indications. (10:25-53) This invention relates generally to two-part polymer com positions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	adhesion to the tissue and gel formation is desired. In particular, they are useful as tissue sealants, in promoting hemostasis, for drug delivery, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions. (Abstract, 1:5-18)
12. The method of claim 1 wherein the electrophilic functional groups of the first precursor comprise n-hydroxysuccinimide ester.	**Wallace '725** A particularly preferred composition for hemostatic applications to actively bleeding 30 tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when compared to compositions having only maleimidyl PEG or 35 succinimidyl PEG alone. (9:29-36) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) In another preferred embodiment, the compounds each 55 have 12 functional groups. Such compounds are formed from reacting a first tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" function- 60 ally activated polymers. An example of such a "12-arm" compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size 65 from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials. (4:8-65) Multifunctionally activated polyalkylene oxides, such as polyethylene glycol, are commercially Available, and are also easily prepared using known methods. For example, see Chapter 22 of Poly(ethylene Glycol) Chemistry: Biotechni cal and Biomedical Applications, J. Milton Harris, ed., Plenum Press, NY (1992); and Shearwater Polymers, Inc. Catalog, Polyethylene Glycol Derivatives, Huntsville, Ala.

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	(1997–1998). For use as a tissue sealant, the preferred combination of activated polymers is as follows: the sulfhydry-reactive group-containing compound is the tet rafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra-succinimidylglutarate (10,000 mol, wt.); and the sulfhydryl group-containing compound is the tetrafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra sulfhydryl (10,000 mol, wt.). In both cases, these "four-arm" PEGs are formed by ethoxylation of pentaerythritol, where each of the four chains is approximately 2,500 mol. wi., and then derivatized to introduce the functional groups onto each of the four arms. Also preferred are analogous poly(ethylene glycol)-like compounds polymerized from di-glycerol instead of pentaerythritol. (5:35-55) For most applications, sulfhydryl reactive groups that react with sulfhydryl groups to form thioester linkages are preferred. Such compounds are depicted in FIG. 1 and include, inter alia, the following compounds, with the numbers in parentheses corresponding to the structures shown in FIG. 1: mixed anhydrides, such as PEG-glutaryl-acetyl-anhydride (1), PEG-glutaryl-isovaleryl-anhydride (2), PEG-glutaryl-pivalyl-anhydride (3) and related compounds as presented in Bodanszky, p. 23; Ester derivatives of phosphorus, such as structures (4) and (5); ester derivatives of p-nitrophenol (6) of p-nitrothiophenol (7), of pentafluorophenol (8), of structure (9) and related active esters as presented by Bodanszky, pp. 31-32, and Table 2; esters of substituted hydroxylamines, such as those of N-hydroxy-phthalimide (10), N-hydroxy-succinimide (11), and N-hydroxy-glutarimide (12), as well as related structures in Bodanszky; Table 3; esters of 1-hydroxybenzotriazole (13), 3-hydroxy-3,4-dihydro-benzotriazine-4-one (14) and 3-hydroxy-3,4-dihydro-quinazoline-4-one; derivatives of carbonylimidazole; and isocyanates. With these compounds, auxiliary reagents can also be used to facilitate bond formation, such as 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide can be used to facilitate coupling of carboxyl groups (i.e., glutarate and succinate) with sulfhydryl groups.
15. The method of claim 1	**Wallace '725**

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
wherein at least one of the precursors is selected to further comprise a chemical group having the formula $(CH_2CH_2O)_n$.	Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) In another preferred embodiment, the compounds each 55 have 12 functional groups. Such compounds are formed from reacting a first tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" function- 60 ally activated polymers. An example of such a "12-arm"

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size 65 from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials. (4:8-65) Multifunctionally activated polyalkylene oxides, such as polyethylene glycol, are commercially Available, and are also easily prepared using known methods. For example, see Chapter 22 of Poly(ethylene Glycol) Chemistry: Biotechni cal and Biomedical Applications, J. Milton Harris, ed., Plenum Press, NY (1992); and Shearwater Polymers, Inc. Catalog, Polyethylene Glycol Derivatives, Huntsville, Ala. (1997–1998). For use as a tissue sealant, the preferred combination of activated polymers is as follows: the sulfhydry-reactive group-containing compound is the tet rafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra-succinimidylglutarate (10,000 mol, wt.); and the sulfhydryl group-containing compound is the tetrafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra sulfhydryl (10,000 mol, wt.). In both cases, these "four-arm" PEGs are formed by ethoxylation of pentaerythritol, where each of the four chains is approximately 2,500 mol. wi., and then derivatized to introduce the functional groups onto each of the four arms. Also preferred are analogous poly(ethylene glycol)-like compounds polymerized from di-glycerol instead of pentaerythritol. (5:35-55) For most applications, sulfhydryl reactive groups that react with sulfhydryl groups to form thioester linkages are preferred. Such compounds are depicted in FIG. 1 and include, inter alia, the following

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	compounds, with the numbers in parentheses corresponding to the structures shown in FIG. 1: mixed anhydrides, such as PEG-glutaryl-acetyl-anhydride (1), PEG-glutaryl-isovaleryl-anhydride (2), PEG-glutaryl-pivalyl-anhydride (3) and related compounds as presented in Bodanszky, p. 23; Ester derivatives of phosphorus, such as structures (4) and (5); ester derivatives of p-nitrophenol (6) of p-nitrothiophenol (7), of pentafluorophenol (8), of structure (9) and related active esters as presented by Bodanszky, pp. 31-32, and Table 2; esters of substituted hydroxylamines, such as those of N-hydroxy-phthalimide (10), N-hydroxy-succinimide (11), and N-hydroxy-glutarimide (12), as well as related structures in Bodanszky; Table 3; esters of 1-hydroxybenzotriazole (13), 3-hydroxy-3,4-dihydro-benzotriazine-4-one (14) and 3-hydroxy-3,4-dihydro-quinazoline-4-one; derivatives of carbonylimidazole; and isocyanates. With these compounds, auxiliary reagents can also be used to facilitate bond formation, such as 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide can be used to facilitate coupling of carboxyl groups (i.e., glutarate and succinate) with sulfhydryl groups. A particularly preferred composition for hemostatic applications to actively bleeding tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when compared to compositions having only maleimidyl PEG or succinimidyl PEG alone. (9:29-36) The appropriate concentration of the compound, and other optional ingredients, in each component, and thus the relative concentration of the matrix components in the final gel matrix, can easily be **optimized to achieve the desired gelation time** and gel strength using routine experimentation. Using the preferred four-arm PEGs described above, the synthetic polymer is generally present at a concentration of 2 to 50% (w/v), and more preferably 10

Claims 1, 6, 7, 12, 15 and 17	Wallace '725

10-25%. (9:3-8)

For example, both amino PEG and sulfhydryl PEG need a basic pH to enhance nucleophilicity. The effects of pH on gel time are discussed below in the Examples. (9:37-44)

Example 5
Effect of Buffer and Reactive Group on Gel Times
A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetrafunctional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show the difference in gel times in

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	25 amino formulations and sulfhydryl formulations in phosphate buffer. In this buffer, an increase in gelation rate is observed for sulfhydryl formulations compared to amino formulations. Experiments C and D show the difference in gelation times in amino formulations and sulfhydryl formu- 30 lations in carbonate buffer. As shown, a decrease in gel time is observed for sulfhydryl formulations in carbonate buffer. In the preferred P/C Buffer, a gel time of 3 seconds is observed. (13:60-14:34) Example 11 Collagen-Containing Compositions Methylated collagen is prepared by the following process: bovine corium collagen is solubilized using pepsin and purified as described in U.S. Pat. No. 4,233,360. This purfied, solubilized collagen is precipitated by neutralization into 0.2M sodium phosphate, pH 7.2. The precipitate is 40 isolated by centrifugation to a final concentration of 70 *mglml*. The material is dried for two days, and then pulverized. Dry methanol containing HCl (to 0.1 N) is added (40 ml) and stirred for four days. Collagen is separated from the acidic methanol, vacuum dried and sterilized by irradiation. The final product is disolved in water at a pH of 3-4. For delivery as a sealant, 10 mg of the methylated collagen, 100 mg of tetra-functional sulfhydryl-PEG, 10,000 mol. wt., and 100 mg of tetra-functional SG PEG, 10,000 mol. wt., are dissolved in water at pH 3-4 to a final volume 50 of 1 ml (first component). The second component is 1 ml of *PIC* Buffer. Each component is placed in a syringe and mixed and sprayed on the desired test site using a dualsyringe delivery system as described in Example 1. **The applied mixture gels in less than 3 seconds**. (17:33-55)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
17. The method of claim 1 wherein the hydrogel is essentially fully degradable in a patient in less than about 90 days.	**Wallace '725** In another aspect of the present invention, the compounds further comprise chain extenders between the polymer core and the functional groups. Such chain extenders can activate or suppress reactivity of the functional groups, and can also be used to provide sites for hydrolysis or degradation. Suitable chain extenders include poly(amino acids), poly (lactones), poly(anhydrides), poly(orthoesters), poly (orthocarbonates), poly(phosphoesters), and enzymatically cleavable peptide groups. (2:38-47) Chain Extenders Functional groups may be directly attached to the compound core, or they may be indirectly attached through a chain extender. Such chain extenders are well known in the art. See, for example, PCT WO 97/22371, which describes "linking groups" that would be suitable for use as chain extenders in the compositions of the present invention. Chain extenders are useful to avoid stearic hindrance problems that are sometimes associated with the formation of direct linkages between molecules. Alternatively, chain extenders may be used to link several multifunctionally activated compounds together to make larger molecules. In a particularly preferred embodiment, the chain extender can also be used to alter the degradative properties of the compositions after administration and resultant gel formation. For example, chain extenders can be incorporated into one or both of the multifunctionally activated polymers to promote hydrolysis, to discourage hydrolysis, or to provide a site for enzymatic degradation. (7:1-20)

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	Chain extenders may provide sites for degradation, i.e., hydrolysable sites. Examples of hydrolysable chain extend ers include, inter alia, alpha-hydroxy acids such as lactic acid and glycolic acid; poly(lactones) such as caprolactone, valerolactone, gamma butyl lactone and p-dioxanone; poly (amino acids); poly(anhydrides) such as glutarate and suc cinate; poly(orthoesters); poly(orthocarbonates) such as tri methylene carbonate; and poly(phosphoesters). Examples of non-degradable chain extenders include, inter alia, succinimide, propionic acid and carboxymethylate. See, for example, PCT WO 99/07417. Examples of enzymatically degradable chain extenders include Leu-Gly-Pro-Ala, which is degraded by collagenase; and Gly-Pro-Lys, which is degraded by plasmin. (7:32-44) A particularly preferred composition for hemostatic applications to actively bleeding 30 tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions produce gels with enhanced biodegradability and superior gel times when compared to compositions having only maleimidyl PEG or 35 succinimidyl PEG alone. (9:29-36) Example 1 Preparation of a Two-Component Tissue Sealant Composition a. First Component Pentaerythritol poly(ethylene glycol)ether tetra succinimidyl glutarate ("SG-PEG") (mol, wt. 10,000) is

Claims 1, 6, 7, 12, 15 and 17	Wallace '725
	dissolved in 0.5 mM sodium phosphate pH 6.0 at a concen tration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydroly sis and should be used within one hour of preparation). b. Second Component Pentaerythritol poly(ethylene glycol)ether tetra sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mm phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention. (11:23-50) Example 2 Surgical Sealing of Arteries The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). (11:51-63)

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TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	-	+
B	fibrillar collagen	-	+
C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400; gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

(col. 13)

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TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations

Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show a mild gross and histological response of fibrillar collagen (Collagen Corporation, Palo Alto, Calif.) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved. Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network. This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus increasing the amount of free amines on the polymeric network. This treatment decreases the

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biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations. (col. 14)

A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 6

Effect Buffers on Gelation Time.

Test	Buffer	Gel Time (Sec)
A	P/C Buffer	8
B	150 mM phosphate	35
C	58 mM phosphate 91 mM sodium chloride	138
D	58 mM phosphate 91 mM borate	<19
E	58 mM phosphate 91 mM AMPSO*	8

*(3[1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxypropane-sulfonic acid

As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable (col. 15)

Exhibit 5

Exhibit G-17: U.S. Pat No. 8,003,705 and Stockman Pub '454

U.S. Pat No. 8,003,705 and Stockman Pub '454

Claims 4, 6, 13, and 19 of asserted U.S. Pat. No. 8,003,705 (the "'3,705 patent") are either anticipated or rendered obvious alone or in combination with the knowledge of a person of ordinary skill in the art ("POSA"), under 35 U.S.C. §§ 102, 103 by U.S. Pat. Pub. No. 2007/0196454 ("Stockman '454"), filed on January 11, 2007, published on August 23, 2007, and issued as U.S. Pat. No. 9,393,344 on July 19, 2016. To the degree that Stockman '454 does not anticipate these claims, the claims would have been obvious to a POSA as further identified in HyperBranch's November 4, 2016 Invalidity Contentions; December 16, 2016 Frist Supplemental Invalidity Contentions; and Second Supplemental Invalidity Contentions.[1]

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
4. A kit comprising: A first biocompatible precursor having at least two electrophilic functional groups, and a second biocompatible precursor comprising at least two primary amine functional groups,	"Kits of the Invention One aspect of the present invention relates to a kit for the preparation of a sealant comprising: a polymerization agent selected from the group consisting of a compound of formula Ia or formula Ib, wherein formulae Ia or Ib are as defined above; and instructions for preparing said sealant. Another aspect of the present invention relates to a kit for the preparation of a sealant comprising: a polymerization agent selected from the group consisting of a compound of formula Ia, formula Ib, and formula Ic, wherein formulae Ia, Ib, and Ic are as defined above; and instructions for preparing said sealant. Another aspect of the present invention relates to a kit for the preparation of a sealant comprising: a compound of formula I and formula III, wherein formulae I and III are as defined above; and instructions for preparing said sealant. Another aspect of the present invention relates to a kit for the preparation of a sealant comprising: a compound of formula Ic, wherein formula Ic is as defined above; and instructions for preparing said sealant. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a compound of formula III, wherein formula III is as defined above. In certain embodiments, the present invention relates to the aforementioned kit, further comprising

[1] The excerpts cited herein are exemplary. For any claim limitation, Defendant may rely on excerpts cited for any other limitation and/or additional excerpts not set forth fully herein to the extent necessary to provide a more fulsome explanation for a reference's disclosure of a limitation. Where an excerpt refers to or discusses a figure or figure items, that figure and any additional descriptions of that figure should be understood to be incorporated by reference as if set forth fully therein. Where an excerpt contains underlining, bold, or italics, the emphasis is only meant as an example and is not intended to be limiting. The absence of citations to a combination reference for any claim element is not an admission that the combination reference does not teach or fairly suggest that claim element.

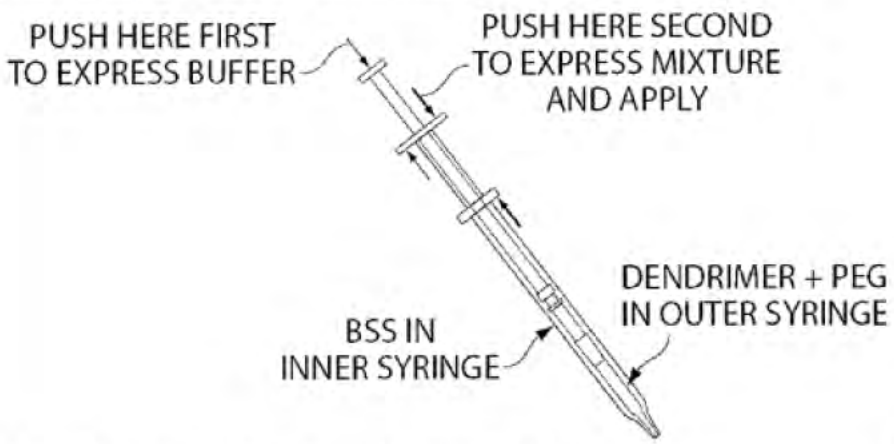
Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	a desiccant. In certain embodiments, the present invention relates to the aforementioned kit, further comprising an antioxidant. In certain embodiments, the present invention relates to the aforementioned kit, wherein said antioxidant is selected from the group consisting of sodium metabisulfite, citric acid, and ascorbic acid. In certain embodiments, the present invention relates to the aforementioned kit, further comprising an inert atmosphere. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit has a sterility assurance level of at least about 10−3. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit has a sterility assurance level of at least about 10−6. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit was sterilized using E-beam or gamma radiation. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit was sterilized using E-beam radiation. In certain embodiments, the present invention relates to the aforementioned kit, wherein said e-beam radiation is between 2 and 100 kGy. In certain embodiments, the present invention relates to the aforementioned kit, wherein said e-beam radiation is between 10 and 80 kGy. In certain embodiments, the present invention relates to the aforementioned kit, wherein said e-beam radiation is between 15 and 40 kGy. In certain embodiments, the present invention relates to the aforementioned kit, wherein said e-beam radiation is between 2 and 40 kGy. In certain embodiments, the present invention relates to the aforementioned kit, wherein said e-beam radiation is between 3 and 20 kGy. In certain embodiments, the present invention relates to the aforementioned kit, wherein said e-beam radiation is between 5 and 12 kGy. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit was sterilized by multiple exposures to E-beam or gamma radiation. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit comprises more than one compound of formula III.

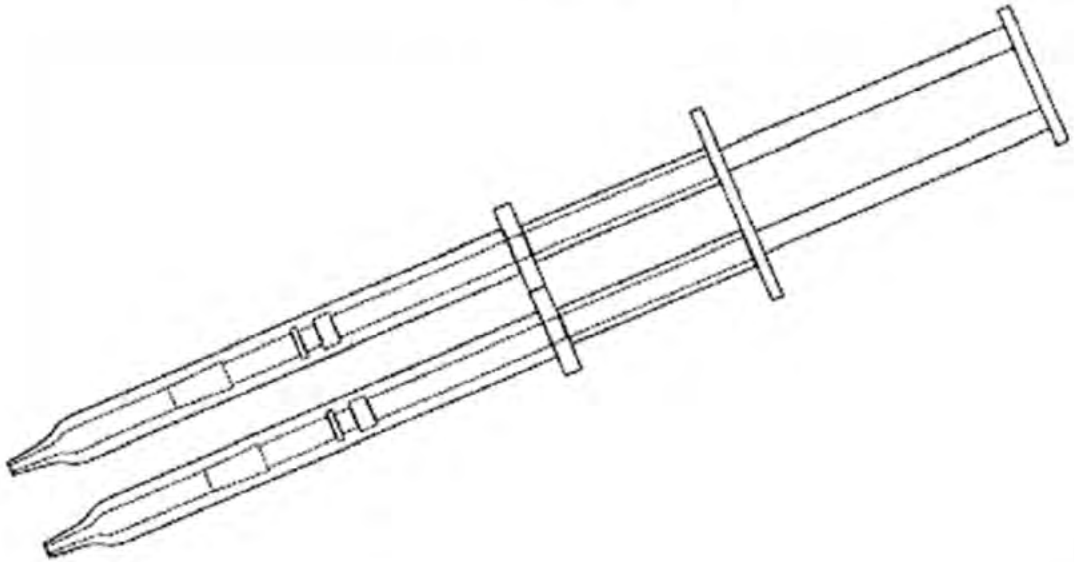
Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit comprises more than one compound of formula Ia or Ib. In certain embodiments, the present invention relates to the aforementioned kit, wherein said kit further comprises a medicament, colorant, flavoring, scent, fibrous additive, thickener or plasticizer. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a moisture-barrier element. The mositure-barrier element may be conditioned for use in the preparation of a solution to be used in a method according to the present invention. Alternatively, a second component of the kit may be contained within the moisture-barrier element. For example, a water-sensitive reagent, such as a PEG-bis(NHS ester), may be contained in a moisture-barrier element, thereby limiting or preventing hydrolysis of the water-sensitive reagent between the manufacture date and the use date of the kit. Further, a kit may contain a plurality of moisture-barrier elements, each of which may be conditioned for use in the same or distinct ways. For example, for a kit containing a plurality of water-sensitive reagents each of them may be contained in an individual moisture-barrier element. Alternatively, a moisture-barrier element may contain a plurality of water-sensitive reagents. A moisture-barrier element may be characterized in a number of ways or a combination of them. For example, a moisture-barrier element may be characterized by its shape (e.g., pouch, vial, sachet, ampule); composition (e.g., glass, foil, Teflon®, stainless steel); and/or it may be characterized by a functional quality (e.g., moisture-vapor transmission rate (MVTR)). MVTR is an important means of characterizing a moisture-barrier element because: those of ordinary skill in the art understand how to measure the MVTR of a material; MVTR values for various materials are known; and the MVTR of a moisture-barrier element quantifies its ability to exclude water from it contents. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a moisture-barrier element with a moisture vapor transmission rate (MVTR) less than or equal to about 0.15 gram per 100 square inches per day. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a moisture-barrier element with a moisture vapor transmission rate (MVTR) less than or equal to about 0.02 gram per 100 square inches per day. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a moisture-barrier element with a moisture vapor transmission rate (MVTR) less than or equal to

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	about 0.15 gram per 100 square inches per day; wherein said moisture-barrier element comprises said polymerization agent selected from the group consisting of a compound of formula Ia and formula Ib. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a moisture-barrier element with a moisture vapor transmission rate (MVTR) less than or equal to about 0.02 gram per 100 square inches per day; wherein said moisture-barrier element comprises said polymerization agent selected from the group consisting of a compound of formula Ia and formula Ib. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a catheter. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a syringe. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a brush. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a spray container and/or an aerosol container. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a device for endoscopic delivery. Endoscopy is a surgical technique that involves the use of an endoscope, a special viewing instrument that allows a surgeon to see images of the body's internal structures through very small incisions. Endoscopic surgery has been used for decades in a number of different procedures, including gallbladder removal, tubal ligation, and knee surgery. An endoscope typically consists of two basic parts: A tubular probe fitted with a tiny camera and bright light, which is inserted through a small incision; and a viewing screen, which magnifies the transmitted images of the body's internal structures. During surgery, the surgeon watches the screen while moving the tube of the endoscope through the surgical area. In certain embodiments, the present invention relates to the aforementioned kit, further comprising a device for laparoscopic delivery. Laparoscopic surgery is a "minimally invasive" surgical technique. Laparoscopy has been used successfully to treat gynecological problems, gallbladder disease, and perform colorectal surgery for many years. The word "laparoscopy" means to look inside the abdominal cavity with a special camera or "scope." Laparoscopy, also known as "keyhole" surgery, has also been used for many years to diagnose medical conditions inside the abdominal cavity.

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	In certain embodiments of the kits, a liquid reagent is contained in a vial, and a powdered reagent is contained in a single-barreled syringe. At time of use, the vial and syringe are placed into liquid communication, and the liquid is withdrawn from the vial into the powder-filled syringe, thereby mixing the two reagents." (¶¶1745-1787) "One aspect of the present invention generally relates to methods of sealing a wound or tissue plane or filling a void splace. In a preferred embodiment, the wound is an ophthalmic, pleural or dural wound. In certain instances, the compositions used to seal the wound or tissue plane comprises a polyalkyleneamine. In a preferred embodiment, the polyalkyleneamine is polyethyleneimine. Treatment of the polyethyleneimine with a cross-linking reagent causes the polyethyleneimine polymers to polymerize forming a seal. In certain instances, the cross-linking reagent is a polyethylene glycol having reactive terminal groups. In certain instances, the reactive terminal groups are activated esters, such as N-hydroxy succinimide ester. In certain instances, the reactive terminal groups are isocyanates. In certain instances, the polyethyleneimine has a lysine, cysteine, isocysteine or other nucleophilic group attached to the periphery of the polymer. In certain instances, the polyethyleneimine is mixed with a second polymer, such as a polyethylene glycol containing nucleophilic groups. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing electrophilic groups with a cross-linking reagent containing nucleophilic groups. In certain instances, the electrophilic groups on the polyalkyleneamine are activated esters, such as N-hydroxy succinimide ester. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing photopolymerizable groups with ultraviolet or visibile light. Compositions used to seal the wound which contain PEI or a derivative of PEI are found to adhere tightly to the tissue. Other aspects of the present invention relate to methods of filling a void of a patient or adhering tissue. In certain instances, the methods use a polyalkyleneamine. In a preferred embodiment, the polyalkyleneamine is polyethyleneimine. Another aspect of the present invention relates to a polymeric composition formed by exposing a polyalkyleneamine to an activated polyalkylene glycol. In certain instances, the composition is attached to mammalian tissue." (Abstract) "In certain instances, the polyethyleneimine has a lysine, cysteine, isocysteine or other nucleophilic group attached to the periphery of the polymer. In certain instances, the polyethyleneimine is mixed

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	with a second polymer, such as a polyethylene glycol containing nucleophilic groups. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing electrophilic groups with a cross-linking reagent containing nucleophilic groups." (¶35) "FIG. 3 depicts polyethylene glycols that may be reacted with nucleophile-bearing poly alkyleneamines to form a hydrogel, wherein variable w is an integer in the range of about 5 to about 200. FIG. 4 depicts polyethylene glycols that may be reacted with nucleophile-bearing poly alkyleneamines to form a hydrogel, wherein variable w is an integer in the range of about 5 to about 200. FIG. 5 depicts poly alkyleneamines that may be reacted with nucleophile-bearing polyalkylene glycols, e.g., PEG-(NH2)2, to form a hydrogel; wherein variables x, y, and z each represent an integer in the range of about 2 to about 200." (¶¶38-40) "In certain instances, the dendritic polymers have nucleophilic groups, such as primary amino groups or thiol groups, which can react with electrophilic groups, such as an acrylate, succinimidyl ester, maleimide, ester aldehyde, or aldehyde on a small molecule. In certain instances, the dendritic polymer has nucleophilic groups capable of reacting with an activated diester of sebabic acid." (¶95) "In certain instances, the polyethylene **glycol cross-linking agent contains two or more different electrophiles**. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90)

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	See also, ¶117; ¶118
a third biocompatible precursor comprising at least two primary amine functional groups and,	"In certain instances, a polyalkylene glycol containing nucleophilic groups is added to the polyalkyleneamine prior to mixing the polyalkyleneamine with a polyalkylene glycol containing electrophilic groups. In certain instances, a PEG is modified to contain amine groups and/or thiol groups. The modified PEG is mixed with the polyalkyleneamine, and then the polyalkyleneamine/modified-PEG solution is added to the PEG-electrophile solution to form the hydrogel. Incorporation of this third active component into the hydrogel can affect hydrogel properties. For example, the resultant hydrogel may swell more, be less mechanically strong, and/or degrade faster compared to a hydrogel prepared without a PEG containing nucleophilic groups." (¶117) "In certain instances, the polyethyleneimine is mixed with a second polymer, such as a polyethylene glycol containing nucleophilic groups." (¶35) See also, abstract
an applicator;	"FIG. 10 depicts a double-acting, single-barrel syringe." (¶45)  (Fig. 10) "FIG. 11 depicts a double-barrel syringe." (¶46)

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	 (Fig. 11) "The materials used to form the sealant of the present invention may be delivered to the wound of a patient using a large number of known delivery devices. For example, the delivery system may be a single-barrel syringe system. In certain instances, the single-barrel syringe is a double acting, single-barrel syringe system as displayed in FIG. 10. In certain situations, a double- or multi-barrel syringe system, as displayed in FIG. 11, may be preferable. In instances where the polymerizable polyalkyleneamine is mixed with a polymerization agent prior to delivering the solution to the wound of a patient, a delivery device that flows two or more streams of liquid in a mixing chamber may be preferable. Alternatively, a delivery device that mixes two solids and two liquids and then separately flows these streams of liquid to a mixing chamber may be advantageous. In certain instances, delivery may be assisted with machines, compressed air or gases, and the like. Of course, variations may be made in the size of the delivery device, the length of the delivery device, and/or the use of machines to aid in delivery." (¶143) "The operative must be careful not to seal the applicator to the tissue or to seal surrounding tissue

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	with glue given its quick reaction." (¶22) See also, ¶132; ¶152; ¶153; ¶1788; Examples 46-47; Examples 52-54;
wherein the first biocompatible precursor, the second biocompatible precursor, and the third biocompatible precursor are reactable with each other to form a crosslinked hydrogel, are resistant to enzymatic degradation, and at least one of the first, second, or third biocompatible precursors comprises at least one isolated hydrolytically degradable ester group;	"One aspect of the present invention generally relates to methods of sealing a wound or tissue plane or filling a void splace. In a preferred embodiment, the wound is an ophthalmic, pleural or dural wound. In certain instances, the compositions used to seal the wound or tissue plane comprises a polyalkyleneamine. In a preferred embodiment, the polyalkyleneamine is polyethyleneimine. Treatment of the polyethyleneimine with a cross-linking reagent causes the polyethyleneimine polymers to polymerize forming a seal. In certain instances, the cross-linking reagent is a polyethylene glycol having reactive terminal groups. In certain instances, the reactive terminal groups are activated esters, such as N-hydroxy succinimide ester. In certain instances, the reactive terminal groups are isocyanates. In certain instances, the polyethyleneimine has a lysine, cysteine, isocysteine or other nucleophilic group attached to the periphery of the polymer. In certain instances, the polyethyleneimine is mixed with a second polymer, such as a polyethylene glycol containing nucleophilic groups. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing electrophilic groups with a cross-linking reagent containing nucleophilic groups. In certain instances, the electrophilic groups on the polyalkyleneamine are activated esters, such as N-hydroxy succinimide ester. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing photopolymerizable groups with ultraviolet or visibile light. Compositions used to seal the wound which contain PEI or a derivative of PEI are found to adhere tightly to the tissue. Other aspects of the present invention relate to methods of filling a void of a patient or adhering tissue. In certain instances, the methods use a polyalkyleneamine. In a preferred embodiment, the polyalkyleneamine is polyethyleneimine. Another aspect of the present invention relates to a polymeric composition formed by exposing a polyalkyleneamine to an activated polyalkylene glycol. In certain instances, the composition is attached to mammalian tissue." (Abstract) "In certain instances, the polyethyleneimine has a lysine, cysteine, isocysteine or other nucleophilic group attached to the periphery of the polymer. In certain instances, the polyethyleneimine is mixed with a second polymer, such as a polyethylene glycol containing nucleophilic groups. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a

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	polyalkyleneamine bearing electrophilic groups with a cross-linking reagent containing nucleophilic groups." (¶35) "FIG. 3 depicts polyethylene glycols that may be reacted with nucleophile-bearing poly alkyleneamines to form a hydrogel, wherein variable w is an integer in the range of about 5 to about 200. FIG. 4 depicts polyethylene glycols that may be reacted with nucleophile-bearing poly alkyleneamines to form a hydrogel, wherein variable w is an integer in the range of about 5 to about 200. FIG. 5 depicts poly alkyleneamines that may be reacted with nucleophile-bearing polyalkylene glycols, e.g., PEG-(NH2)2, to form a hydrogel; wherein variables x, y, and z each represent an integer in the range of about 2 to about 200." (¶¶38-40) "In certain instances, the dendritic polymers have nucleophilic groups, such as primary amino groups or thiol groups, which can react with electrophilic groups, such as an acrylate, succinimidyl ester, maleimide, ester aldehyde, or aldehyde on a small molecule. In certain instances, the dendritic polymer has nucleophilic groups capable of reacting with an activated diester of sebabic acid." (¶95) "In certain instances, the polyethylene **glycol cross-linking agent contains two or more different electrophiles**. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63)

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	"Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine,

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	the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶117; ¶118; ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
wherein the applicator is configured to mix at least the first precursor, the second precursor, and the third precursor to form a crosslinked hydrogel in situ comprising covalent bonds formed by reaction of the functional groups of the precursors and further comprising the at least one isolated hydrolytically degradable ester group;	"Alternatively, the polymers and cells can be mixed and then injected into the in vivo site and crosslinked in situ for tissue repair or replacement. The crosslinked polymers provide a three dimensional templates for new cell growth." (¶124) Example 48 In Vivo Application of PEI:PEG-NHS gel systems. A single barreled syringe was filled with 36 mg of 3400 molecular weight PEG-NHS powder. A separate vial was filled with 220 µl of an aqueous buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, 5 mM sodium meta-bisulfite, and 125 mM sodium phosphate monobasic) containing 2000 molecular weight PEI (15% total solids 15:1 PEG:PEI Ratio (w/w)). A 20 gauge cannula was attached to the luer lock syringe and the tip of the cannula was placed into the buffer filled vial. The plunger of the syringe was pulled backward to draw the buffer solution into the powder filled syringe. The plunger was then pushed forward and the contents of the syringe was expressed back into the vial. The process was repeated two additional times in order to thoroughly mix the powder and liquid components. The final solution was then applied through the cannula onto the cornea of a rabbit in which a 3.0 mm linear incision had been made. The resulting application of sealant was found to be approximately 1.5 mm thick and resulted in effectively sealing the incision. (¶1900) Example 49 In Vivo Application of PEI:PEG-NHS gel systems. A single barreled syringe was filled with 36 mg of 3400 molecular weight PEG-NHS powder. A separate vial was filled with 220 µl of an aqueous buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, 5 mM sodium meta-bisulfite, and 125 mM sodium phosphate monobasic) containing 2000 molecular weight PEI (15% total solids 15:1 PEG:PEI Ratio (w/w)). A 20 gauge cannula was attached to the luer lock syringe and the tip of the cannula was placed into the buffer filled vial. The plunger of the syringe was pulled backward to draw the buffer solution into the powder filled syringe. The plunger was then pushed forward and the contents of the syringe was expressed back

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	into the vial. The process was repeated two additional times in order to thoroughly mix the powder and liquid components. The final solution was then applied onto the cornea of a rabbit in which a 3.0 mm linear incision using a small, nylon bristled paint brush. The resulting application of sealant was found to be approximately 0.3 mm thick and resulted in effectively sealing the incision. (¶1901) "The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically.

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	Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63) "Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic

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	acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine, the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
wherein the hydrogel comprises a sufficient number of the at least one isolated hydrolytically degradable ester groups in the crosslinked hydrogel so that the crosslinked hydrogel is degradable in less than about 180 days, is resistant to enzymatic degradation, and is degradable by hydrolysis of the at least one isolated hydrolytically degradable ester group; and	"The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters.

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	Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63) "Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and

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	a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine, the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
wherein the kit further comprises instructions that comprise directions for making a hydrogel that is degradable in an amount of time, with the amount of time being less than about 180 days.	"One aspect of the present invention relates to a kit for the preparation of a sealant comprising: a polymerization agent selected from the group consisting of a compound of formula Ia or formula Ib, wherein formulae Ia or Ib are as defined above; and instructions for preparing said sealant. Another aspect of the present invention relates to a kit for the preparation of a sealant comprising: a polymerization agent selected from the group consisting of a compound of formula Ia, formula Ib, and formula Ic, wherein formulae Ia, Ib, and Ic are as defined above; and instructions for preparing said sealant. Another aspect of the present invention relates to a kit for the preparation of a sealant comprising: a compound of formula I and formula III, wherein formulae I and III are as defined above; and instructions for preparing said sealant. Another aspect of the present invention relates to a kit for the preparation of a sealant comprising: a compound of formula Ic, wherein formula Ic is as defined above; and instructions for preparing said sealant." (¶¶1746-1755) "The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable

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	of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63)

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	"Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine,

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
6. The kit of claim 4 wherein the amount of time is less than about 90 days.	"The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and

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	no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63) "Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of

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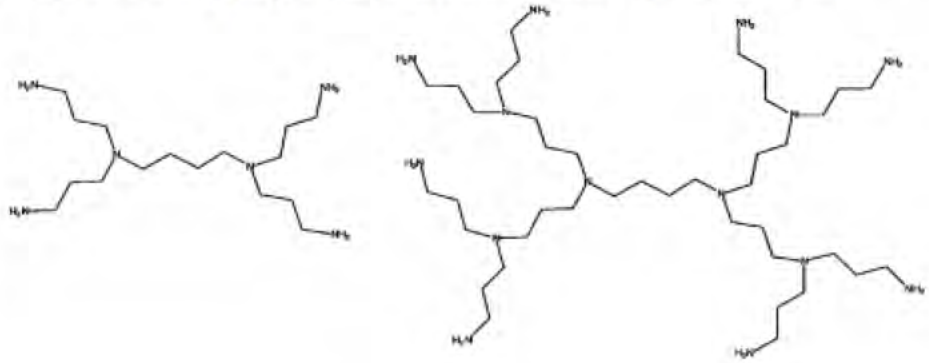
	about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine, the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
19. The kit of claim 4 wherein the second biocompatible precursor is a member of the group consisting of tetraethylenepentamine, pentaethylenehexamine, methylenebis(methylcyclohexamine), diaminocyclohexane, n-(2-aminoethyl)-1,3-propanediamine, diaminomethyldipropylamine, and iminobispropylamine.	"FIG. 1 a and FIG. 1 b both depict poly alkyleneamines that may be reacted with electrophile-bearing polyalkylene glycols to form a hydrogel." (¶36) 

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	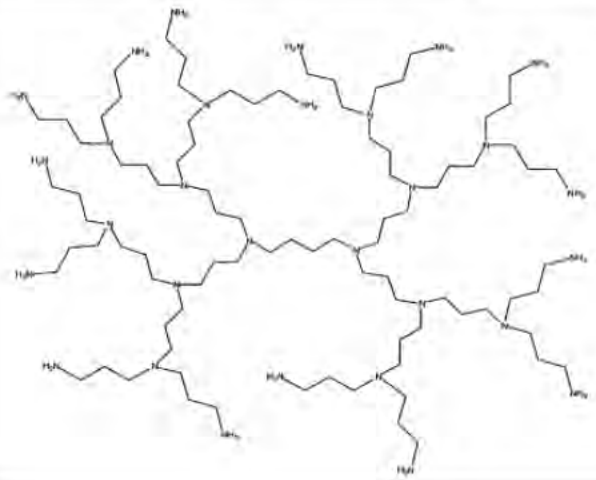

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	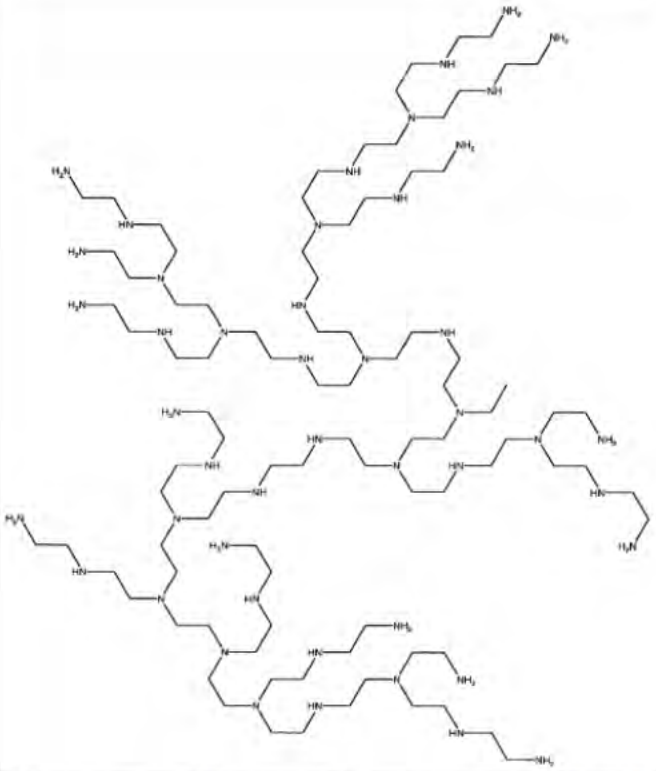
11. A method of making a hydrogel, the method comprising: providing a first biocompatible precursor having at least two electrophilic functional groups, a second biocompatible precursor comprising at least two	"One aspect of the present invention generally relates to methods of sealing a wound or tissue plane or filling a void splace. In a preferred embodiment, the wound is an ophthalmic, pleural or dural wound. In certain instances, the compositions used to seal the wound or tissue plane comprises a polyalkyleneamine. In a preferred embodiment, the polyalkyleneamine is polyethyleneimine. Treatment of the polyethyleneimine with a cross-linking reagent causes the polyethyleneimine polymers to polymerize forming a seal. In certain instances, the cross-linking reagent is a polyethylene glycol having reactive terminal groups. In certain instances, the reactive terminal groups are activated esters, such as N-hydroxy succinimide ester. In certain instances, the reactive terminal groups are isocyanates. In certain instances, the polyethyleneimine has a lysine, cysteine, isocysteine or other nucleophilic group attached to the periphery of the polymer. In certain

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primary amine functional groups, a third biocompatible precursor comprising at least two primary amine functional groups;	instances, the polyethyleneimine is mixed with a second polymer, such as a polyethylene glycol containing nucleophilic groups. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing electrophilic groups with a cross-linking reagent containing nucleophilic groups. In certain instances, the electrophilic groups on the polyalkyleneamine are activated esters, such as N-hydroxy succinimide ester. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing photopolymerizable groups with ultraviolet or visibile light. Compositions used to seal the wound which contain PEI or a derivative of PEI are found to adhere tightly to the tissue. Other aspects of the present invention relate to methods of filling a void of a patient or adhering tissue. In certain instances, the methods use a polyalkyleneamine. In a preferred embodiment, the polyalkyleneamine is polyethyleneimine. Another aspect of the present invention relates to a polymeric composition formed by exposing a polyalkyleneamine to an activated polyalkylene glycol. In certain instances, the composition is attached to mammalian tissue." (Abstract) "In certain instances, the polyethyleneimine has a lysine, cysteine, isocysteine or other nucleophilic group attached to the periphery of the polymer. In certain instances, the polyethyleneimine is mixed with a second polymer, such as a polyethylene glycol containing nucleophilic groups. In certain instances, the compositions used to seal the wound or tissue plane are formed by reacting a polyalkyleneamine bearing electrophilic groups with a cross-linking reagent containing nucleophilic groups." (¶35) "FIG. 3 depicts polyethylene glycols that may be reacted with nucleophile-bearing poly alkyleneamines to form a hydrogel, wherein variable w is an integer in the range of about 5 to about 200. FIG. 4 depicts polyethylene glycols that may be reacted with nucleophile-bearing poly alkyleneamines to form a hydrogel, wherein variable w is an integer in the range of about 5 to about 200. FIG. 5 depicts poly alkyleneamines that may be reacted with nucleophile-bearing polyalkylene glycols, e.g., PEG-(NH2)2, to form a hydrogel; wherein variables x, y, and z each represent an integer in the range of about 2 to about 200." (¶¶38-40)

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	"In certain instances, the dendritic polymers have nucleophilic groups, such as primary amino groups or thiol groups, which can react with electrophilic groups, such as an acrylate, succinimidyl ester, maleimide, ester aldehyde, or aldehyde on a small molecule. In certain instances, the dendritic polymer has nucleophilic groups capable of reacting with an activated diester of sebabic acid." (¶95) "In certain instances, the polyethylene **glycol cross-linking agent contains two or more different electrophiles**. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) See also, ¶117; ¶118
wherein at least one of the first, the second, or the third biocompatible precursors comprises at least one isolated hydrolytically degradable ester group; wherein the first, the second, and the third biocompatible precursors are resistant to enzymatic degradation; and	"The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain.

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63) "Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
	electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine, the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
mixing, in situ, the first biocompatible precursor, the second biocompatible precursor, and the third biocompatible precursor to form a crosslinked hydrogel that comprises covalent bonds formed by reaction of the	"Alternatively, the polymers and cells can be mixed and then injected into the in vivo site and crosslinked in situ for tissue repair or replacement. The crosslinked polymers provide a three dimensional templates for new cell growth." (¶124) Example 48 In Vivo Application of PEI:PEG-NHS gel systems. A single barreled syringe was filled with 36 mg of 3400 molecular weight PEG-NHS powder. A separate vial was filled with 220 µl of an aqueous buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
functional groups of the first, the second, and the third biocompatible precursors, with the hydrogel being resistant to enzymatic degradation and comprising the at least one isolated hydrolytically degradable ester group; wherein the hydrogel comprises a sufficient number of the at least one isolated hydrolytically degradable ester groups in the crosslinked hydrogel so that the crosslinked hydrogel is degradable in less than about 180 days and is degradable by hydrolysis of the at least one isolated hydrolytically degradable ester group.	decahydrate, 5 mM sodium meta-bisulfite, and 125 mM sodium phosphate monobasic) containing 2000 molecular weight PEI (15% total solids 15:1 PEG:PEI Ratio (w/w)). A 20 gauge cannula was attached to the luer lock syringe and the tip of the cannula was placed into the buffer filled vial. The plunger of the syringe was pulled backward to draw the buffer solution into the powder filled syringe. The plunger was then pushed forward and the contents of the syringe was expressed back into the vial. The process was repeated two additional times in order to thoroughly mix the powder and liquid components. The final solution was then applied through the cannula onto the cornea of a rabbit in which a 3.0 mm linear incision had been made. The resulting application of sealant was found to be approximately 1.5 mm thick and resulted in effectively sealing the incision. (¶1900) Example 49 In Vivo Application of PEI:PEG-NHS gel systems. A single barreled syringe was filled with 36 mg of 3400 molecular weight PEG-NHS powder. A separate vial was filled with 220 µl of an aqueous buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, 5 mM sodium meta-bisulfite, and 125 mM sodium phosphate monobasic) containing 2000 molecular weight PEI (15% total solids 15:1 PEG:PEI Ratio (w/w)). A 20 gauge cannula was attached to the luer lock syringe and the tip of the cannula was placed into the buffer filled vial. The plunger of the syringe was pulled backward to draw the buffer solution into the powder filled syringe. The plunger was then pushed forward and the contents of the syringe was expressed back into the vial. The process was repeated two additional times in order to thoroughly mix the powder and liquid components. The final solution was then applied onto the cornea of a rabbit in which a 3.0 mm linear incision using a small, nylon bristled paint brush. The resulting application of sealant was found to be approximately 0.3 mm thick and resulted in effectively sealing the incision. (¶1901) "The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a

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	Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63) "Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention

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	maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine, the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;
13. The method of claim 11	"The PAI derivatives may contain hydrolytically and/or enzymatically degradable linkages capable

Claims 4, 6, 11, 13, and 19	Stockman Pub '454
wherein the hydrogel is degradable in less than about 90 days.	of releasing the functional derivatives, active agents, pharmaceutical agents, preservatives, radio isotopic ions, magnetically detectable ions, antibodies, colorants, dyes, or other visualization agents." (¶0077) "Example 63 Tissue Ingrowth Studies. This study evaluated the biodegradation of hydrogel formulations in a Sprague-Dawley rat model at timepoints up to 10 weeks post application. Thirty two male Sprague-Dawley rats were anesthetized with isoflurane. The test sites were shaved and cleaned with alcohol and Betadine scrub. Betadine solution was applied and each rat was opened using scissors. Four hydrogel formulations were applied by subcutaneous spray application in two areas in each rat on Day 1. Each rat was closed using 4.0 suture material and treated with Buprenorphine for pain. The four formulations tested containing either 1) PEG3400-SPA 15 wt % 2) PEG3400-SPA 7.5 wt % 3) PEG10000-SG/PEG3400-SPA 20-80 blend (w/w) or 4) PEG10000-SPA 15 wt %. Each activated PEG-NHS powder was dissolved in a phosphate monobasic buffer (60 to 120 mM) and was mixed at time of use with a buffer solution (approximately 25 mM sodium bicarbonate, 25 mM sodium tetraborate decahydrate, and 5 mM sodium meta-bisulfite) containing 2000 molecular weight PEI to produce a hydrogel with 15% total solids and a PEG:PEI Ratio (w/w) so that the theoretical number of primary amines was equal to the number of activated esters. Two animals per formulation group were sacrificed by carbon dioxide asphyxiation at 1, 2, 8, and 10 weeks post-op. Necropsy of area of application was performed. All excised tissues were histologically processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Upon examination, it was noted that all 4 formulations appeared similar histologically at all time points. All formulations showed minimal inflammatory response with no fibrous encapsulation and no foreign body reaction (FIGS. 35 and 36). All formulations also showed cell migration, tissue ingrowth, and vascularization (FIGS. 35 and 36). Degradation, evidenced by foamy macrophages, was evident within each formulation, but at least a portion of the hydrogel material was observed throughout the ten week study." (Example 63)

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	"Besides an easy and fast application on the wound, the characteristics of an adhesive include: 1) bind to the tissue (necrosed or not, very often wet) with an adequate adhesion force; 2) be non-toxic; 3) be biodegradable or resorbable; 4) be sterilizable; and 5) not interfere with the healing process." (¶6) "Accordingly, in certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 7 days. In certain instances, the polyalkyleneamine hydrogel sealants of the invention maintain mechanical strength for at least about 20 days. This rate of degradation allows native tissue to ingrow and replace the hydrogel as it degrades." (¶83) "In certain instances, the polyethylene glycol cross-linking agent contains two or more different electrophiles. The different electrophiles may have similar or dissimilar reactivities. The different electrophiles provide linkages having similar or dissimilar degradation rates imparting. The selection of electrophiles allows for control over the crosslinking reactions to form the hydrogels, the adhesive properties, and the degradation rate of the formed hydrogel. For example, a polyethylene glycol can be derivatized such that one end of the polyethylene glycol contains a SPA and another end contains a SG. In this example, both are activated esters, but the degradation rates of the two linkages are different. For example, a hydrogel prepared with only a PEG-SPA is generally stable at 37° C. for more than about four months, whereas a hydrogel prepared with PEG-SG is often stable for less than about one week. Notably, one hydrogel prepared from PEI and a PEG-SPA/SG having a 60:40 ratio of SPA:SG degraded in about a week." (¶90) "Another aspect of the invention relates to a composition comprising a degradable scaffold and a hydrogel adhesive. In certain instances, the degradable scaffold comprises a biodegradable polymer. In certain instances, the degradable scaffold comprises poly(glycolic acid), poly(lactic acid), or copolymers thereof. In certain instances, the degradable scaffold comprises poly(lactic acid). In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 500,000 g/mol. In certain instances, the biodegradable polymer has a weight average molecular weight of about 500 g/mol to about 100,000 g/mol. In certain instances, the scaffold is placed in the wound site and the hydrogel adhesive is then applied to the wound. This approach provides that the tissue and the scaffold are secure in the wound site. Alternatively, the scaffold is coated with one component of the hydrogel adhesive, e.g., the polyalkyleneamine,

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	the scaffold is placed in vivo, and then the second component of the hydrogel adhesive is added to form the hydrogel." (¶122) See also, ¶127; 116; ¶165; ¶1561; Example 8; Example 11; Example 55;

EXHIBIT 6

FULLY REDACTED

Exhibit 7

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

INTEGRA LIFESCIENCES CORP., INTEGRA LIFESCIENCES SALES LLC, CONFLUENT SURGICAL, INC., AND INCEPT LLC, Plaintiffs, v. HYPERBRANCH MEDICAL TECHNOLOGY, INC., Defendant.	C.A. No. 15-819-LPS-CJB

PLAINTIFFS' OBJECTIONS AND ANSWERS
TO HYPERBRANCH'S FIRST SET OF INTERROGATORIES (NOS. 1-7)

Pursuant to Rules 26 and 33 of the Federal Rules of Civil Procedure, the Local Rules for the U.S. District Court for the District of Delaware, and subject to their rights to supplement these objections later in discovery, Plaintiffs Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical, Inc., and Incept LLC (collectively "Plaintiffs," as well as "Integra," "Integra Sales," "Confluent," and "Incept," respectively) hereby object to Defendant HyperBranch Medical Technology's ("HyperBranch") First Set of Interrogatories served on September 23, 2016, including each and every definition, instruction, and interrogatory contained therein (collectively "HyperBranch's First Set of Interrogatories"). The fact that Plaintiffs provide an answer to an interrogatory does not constitute an admission or acknowledgement that the interrogatory is proper, that the answers sought are within the bounds of discovery, or that requests for similar information will be treated in a similar fashion. Plaintiffs do not waive any objection by producing such documents, things, or answers, and Plaintiffs reserve the right to continue investigating these matters, to supplement their objections, and to object to future discovery on the same or related matters. Plaintiffs further reserve the

right to object to the admissibility of any answer produced pursuant to these interrogatories, in whole or in part, on any ground including without limitation materiality, relevance, and privilege.

GENERAL OBJECTIONS

Plaintiffs incorporate by reference their General Objections and Objections to Specific Definitions to HyperBranch's Requests for Production. Each of these General Objections is incorporated into the specific objections set forth below, whether or not separately set forth therein.

1. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks to impose upon Plaintiffs any obligation or responsibility broader than, different from, or in addition to those obligations and requirements mandated by the Federal Rules of Civil Procedure, the Federal Rules of Evidence (collectively, the "Federal Rules"), and the Local Rules for the United States District Court for the District of Delaware (the "Local Rules").

2. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs do not intend to produce such privileged or protected documents or information. To the extent that any document or information which is properly subject to any such privilege or protection is inadvertently produced in connection with an answer to an interrogatory, such inadvertent disclosure is not to be construed as a waiver of such privilege or protection, and such document and information, and all copies thereof, shall be returned to counsel for Plaintiffs, in accordance with Fed. R. Evid. 502(b), Fed. R. Civ. P. 26(b)(5)(B), and any relevant Order entered by the Court. Further,

Plaintiffs will limit their privilege log to pre-lawsuit privileged or protected documents or information, if any exist.

3. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent they contain misstatements of fact and/or inaccurate assumptions. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it is overly broad, unduly burdensome, or oppressive. Plaintiffs further object to each and every definition, instruction, and interrogatory to the extent it calls for information that is irrelevant to any claim or defense in this action.

4. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks information already in the possession, custody, or control of HyperBranch as being overly broad, unduly burdensome, expensive, and inconsistent with the Federal Rules.

5. Plaintiffs object to each and every definition, instruction, and interrogatory as being unduly burdensome to the extent it seeks facts, documents, and/or information that is publicly available, unreasonably cumulative or duplicative, or already known and equally available to HyperBranch.

6. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it is vague, ambiguous, fails to describe the information sought with the required reasonable particularity, or is so unintelligible that Plaintiffs cannot ascertain what information is responsive.

7. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it seeks to impose upon Plaintiffs an obligation to investigate or discover information, materials, or documents from any entity other than Plaintiffs, including, but not limited to, third parties or non-parties.

8. Plaintiffs' agreement to furnish information in response to HyperBranch's Interrogatories shall not be deemed to constitute an admission as to its relevancy, nor is it intended to waive any right to object to its admissibility at trial.

9. Plaintiffs object to each interrogatory that requests "each," "every," or "all" (and to similar overly broad terms) information or documents as overbroad and unduly burdensome. Plaintiffs will undertake a diligent and reasonable investigation to gather information in their possession, custody, or control that is responsive to the non-objectionable portions of each interrogatory.

10. Plaintiffs object to each and every definition, instruction, and interrogatory to the extent it contains subparts, is compound and conjunctive, and is otherwise inconsistent with or exceeds the number of interrogatories permitted by any relevant Order entered by the Court. The Court has set a limit of 25 interrogatories for each side. In answering any or all of these Interrogatories or subparts, Plaintiffs do so without waiver of their right to object to and refuse to answer any future Interrogatories on the grounds that such Interrogatories are in excess of the number permitted by the Federal and Local Rules and the Court's Scheduling Order.

11. In addition to these General Objections, Plaintiffs have specific objections as set forth below. By stating these specific objections, Plaintiffs do not waive any of the General Objections that may also be applicable to specific interrogatories.

OBJECTIONS TO SPECIFIC DEFINITIONS

1. Plaintiffs object to the definition of the terms "Plaintiffs," "You," and "Yours" to the extent those terms are overly broad and purport to require Plaintiffs to provide information and/or documents that are not currently within their possession, custody, or control. Plaintiffs object to the definitions of the terms "Plaintiffs," "You," and "Yours" as seeking the disclosure

of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law, in that the definitions specifically cover "attorneys."

2. Plaintiffs object to the definition of "Accused Products" as overbroad, unduly burdensome, and irrelevant to any issue in this matter as "any and all products, activities, services, processes, systems, apparatuses, or things that Plaintiffs accuse of infringing the Asserted Patents in this Action, including Adherus Autospray Dural Sealant, Adherus Dural Sealant, and Adherus Spinal Sealant" include information, products, and/or documents that are not currently within the possession, custody, or control of Plaintiffs. Indeed, this definition explicitly includes documents and things which are in the exclusive control of Defendant and Third Parties.

3. Plaintiffs object to the definition of the term "each" to the extent that the definition purports to impose a meaning broader than the definition provided in the Federal Rules.

4. Plaintiffs object to the definition of "Prior Art" as overbroad, unduly burdensome, and irrelevant to any issue in this matter as "all things, patents, publications, disclosures, sales, or other acts or occurrences included within the broadest meaning of 35 U.S.C. § 102 (or any subpart thereof) and 35 U.S.C. § 103" and "publications, patents, patent applications, inventions by others, uses, sales or offers for sale, and disclosures" purports to require Plaintiffs to provide information and/or documents that are not currently within their possession, custody, or control.

OBJECTIONS AND ANSWERS TO SPECIFIC INTERROGATORIES

INTERROGATORY NO. 1 [9]. On a claim-by-claim basis for each and every claim of the Asserted Patents, identify each individual who You contend contributed to the conception of the invention set forth in each claim, including all supporting facts and evidence of the contribution to the conception of each claim by the identified individual(s) and the dates of such contribution(s).

OBJECTION AND ANSWER TO INTERROGATORY NO. 1 [9]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this interrogatory to the extent it purports to be HyperBranch's first interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's ninth interrogatory. Plaintiffs further object to this interrogatory as being unreasonably cumulative or duplicative, or already known to HyperBranch. *See* Interrogatory No. 1 served by HyperBranch on October 23, 2015. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome in that it requests identification of "all supporting facts and evidence of the contribution to the conception of each claim." Plaintiffs further object to this interrogatory as premature and irrelevant to the extent it is a contention interrogatory that seeks to impose a burden on Plaintiffs to provide a rebuttal position on conception of the inventions claimed in the patents-in-suit prior to the provision of any contention of invalidity of the claims that Defendant is required to provide on November 4, 2016. Validity, including validity of conception and proper inventorship is presumed by the issuance of the patent. Defendant bears the burden of establishing through its invalidity contentions that there is an issue as to validity that would require Plaintiffs to prove an earlier date of invention or confirm the contribution of a listed inventor to the claims of the patents-in-suit. To date, Defendants validity contentions have not met that burden. Plaintiffs

also object to this interrogatory to the extent it calls for legal argument and/or expert testimony, which Plaintiffs may provide, in due course and in accordance with the Court's Scheduling Order.

Subject to and without waiving its objections, Plaintiffs incorporate by reference their response to Interrogatory No. 1 served on November 13, 2015 and all supplements thereto and the Rebuttal Expert Report of Dr. Jimmy Mays and further respond that based on present information Chandrashekhar P. Pathak, Amarpreet S. Sawhney, and Peter G. Edelman contributed to the conception of one or more claims of the '034 Patent, the '406 Patent, the '5,705 Patent, the '566 Patent and the '418 Patent. Plaintiffs further respond that based on present information Amarpreet S. Sawhney, Steven Bennett, and Peter G. Edelman contributed to the conception of one or more claims of the '3,705 Patent. Defendants' present invalidity contentions do not place in dispute the conception or the named inventor's individual contributions to conception of any of the claims. Accordingly, Plaintiffs presently intend to rely on the effective filing date for each of patents-in-suit (including those patents and patent applications to which priority is claimed), including any evidence presented during prosecution of the patents-in-suit (including those patents and patent applications to which priority is claimed), the recitation of the named inventors on the face of each of the patents-in-suit, and the prior sworn deposition testimony (including exhibits used in those depositions) in this matter of the named inventors to identify the dates and individuals contributing to the conception of each of the claims of the patents-in-suit and the prior sworn testimony and multiple expert reports, rebuttal expert reports, and/or declarations of Dr. Jimmy Mays that have previously been provided in this matter. Plaintiffs further respond that they have produced non-privileged documents pursuant to Federal Rule of Civil Procedure 33(d) (including the patents-in-suit, the

patents and applications from which the patents-in-suit claim priority, the prosecution histories of these patents and patent applications, and the laboratory notebooks and the reports summarizing the laboratory work and notebooks of the inventors and individuals working under their direction (*See, e.g.,* Experimental Reports or Technical Documents having an ER[###] or TD-[###] identification)) from which HyperBranch may derive or ascertain information responsive to this interrogatory. Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery or as Defendant's invalidity contentions are fully and completely provided, in accordance with the Rules.

INTERROGATORY NO. 2 [10]. On a claim-by-claim basis for each and every claim of the Asserted Patents, identify what You contend to be the effective filing date for the claim, including all supporting facts and evidence for the identified effective filing date such as, without limitation, the specific page and lines of any prior filed applications that you contend supports Your identified effective filing date for each claim.

OBJECTION AND ANSWER TO INTERROGATORY NO. 2 [10]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this interrogatory to the extent it purports to be HyperBranch's second interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's tenth interrogatory. Plaintiffs further object to this interrogatory as being unreasonably cumulative or duplicative, or already known to HyperBranch. *See* Plaintiffs' Responses and Supplemental Responses to Interrogatory Nos. 1 and 8 and Rebuttal Expert Report of Dr. Jimmy Mays, hereby incorporated by reference in their entirety. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure

of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome and premature at this stage of the litigation in that it requests identification of "all of the factual and legal bases for that contention, and identify all documents and evidence you claim supports that contention." ." Plaintiffs further object to this interrogatory as premature and irrelevant to the extent it is a contention interrogatory that seeks to impose a burden on Plaintiffs to provide a rebuttal position on the effective filing date of each claim prior to the disclosure of any invalidity contention by the Defendant that puts at issue the effective filing date of any claim on which Defendant has the burden of proof and is required to provide its full and complete invalidity contentions. Validity of the claims is presumed by the issuance of the patent. Defendant bears the burden of establishing through its invalidity contentions that there is an issue as to validity that would require Plaintiffs to prove an earlier effective filing date. To date, Defendants validity contentions have not met that burden. Plaintiffs further object to this Interrogatory to the extent it contains subparts which, together with the other Interrogatories, exceed the limit under the Federal Rules. Plaintiffs also object to this interrogatory to the extent it calls for legal argument and/or expert testimony, which Plaintiffs may provide, in due course and in accordance with the Court's Scheduling Order.

Subject to and without waiving its objections, Plaintiffs rely on the disclosures provided in the patents-in-suit including the related U.S. applications provided on the front of each of the patents in suit to provide an effective filing date for each of the claims. Particularly, the related U.S. applications listed on the face of the patents-in-suit show that the effective filing date for many of the limitations found in the claims of the patents-in-suit may extend back to at least as

early as December 4, 1998 and possibly as early as September 23, 1996. For example, many of the limitations claimed in the patents-in-suit can expressly be found in the text of the related U.S. applications. (*See, e.g.*, visualization agent, precursors, biodegradable polymers, biodegradable polymeric crosslinkers, nucleophilic functional groups, electrophilic functional groups, hydrogel film thickness, and many others). Plaintiffs further respond that they have produced non-privileged documents pursuant to Federal Rule of Civil Procedure 33(d) for which the burden of deriving or ascertaining the answer will be substantially the same for HyperBranch as it is for plaintiffs, namely the patents-in-suit, the patents and applications from which the patents-in-suit claim priority, and prosecution histories of these patents and patent applications.

Plaintiffs also identify Exhibits 57 and 58 to the previous deposition of the inventors along with the transcripts of those depositions (i.e., Amar Sawhney and Steven Bennett) as providing further information related to the effective filing date of the claims of the patents-in-suit. *See, e.g.*, Steve Bennett deposition transcript at pp. 147-48.

Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery and as rebuttal if Defendant meets its burden of setting forth a preliminary contention of invalidity that puts at issue the effective filing date of one or more claims of the patents-in-suit in accordance with the rules and the Scheduling Order in this matter..

INTERROGATORY NO. 3 [11]. On a claim-by-claim basis, describe in detail the complete basis for Your contention that each Asserted Claim is not invalid in view of Defendant's invalidity contentions.

OBJECTION AND ANSWER TO INTERROGATORY NO. 3 [11]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this

interrogatory to the extent it purports to be HyperBranch's third interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's eleventh interrogatory. Plaintiffs further object to this interrogatory as being unreasonably cumulative or duplicative, or already known to HyperBranch. *See* Response to HyperBranch Interrogatory Nos. 4 and 7 and Rebuttal Expert Report of Dr. Jimmy Mays. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome and premature at this stage of the litigation in that it requests identification of "describe in detail the complete basis for Your contention." Plaintiffs further object to this interrogatory as premature, irrelevant, overbroad, and unduly burdensome to the extent it is a contention interrogatory that seeks to impose a burden on Plaintiffs to provide a rebuttal position on the validity of each claim prior to the disclosure of any invalidity contention by the Defendant that puts at issue the validity of the claim which Defendant has the burden of proof and is required to provide its full and complete invalidity contentions. Validity of the claims is presumed by the issuance of the patent. Defendant bears the burden of establishing through its invalidity contentions that there is an issue as to validity that would require Plaintiffs to prove a rebuttal position. To date, Defendants validity contentions have not met that burden. Plaintiffs also object to this interrogatory as premature, irrelevant, overbroad, and unduly burdensome as Defendant's present invalidity contentions do not provide the complete factual basis for its invalidity contentions for which it bears the burden of proof. Plaintiffs also object to this

interrogatory to the extent it calls for legal argument and/or expert testimony, which Plaintiffs may provide, in due course and in accordance with the Court's Scheduling Order.

Investigation of the facts is ongoing and the Defendants have not provided their contentions sufficient to put at issue the presumption of validity accorded the claims of a duly issued patent. Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery and in rebuttal to any properly asserted contention of invalidity initially raised by Defendants, to which it has the burden of proof, as required by the Rules and the Scheduling Order in this matter.

INTERROGATORY NO. 4 [12]. Describe in detail all rights that have been held in the Asserted Patents, including a description of the histories of such rights, the persons or entities holding such rights, and all agreements and other documents reflecting such rights (identified by Bates numbers).

OBJECTION AND ANSWER TO INTERROGATORY NO. 4 [12]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this interrogatory to the extent it purports to be HyperBranch's fourth interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's twelfth interrogatory. Plaintiffs also object to this interrogatory to the extent it is overly broad and unduly burdensome as being duplicative of previous HyperBranch Interrogatory No. 5. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome in that it requests that Plaintiffs "[d]escribe in

detail all rights that have been held in the Asserted Patents . . . and all agreements and other documents reflecting such rights."

Subject to and without waiving its objections, Plaintiffs respond by incorporating by reference in their entirety the previous responses and supplements thereto to HyperBranch Interrogatory No. 5. The original rights in the earliest priority documents set forth on the face of the patents-in-suit resided with Mr. Chandrashekhar P. Pathak and commenced as of the filing dates of each of the respective filing dates of the provisional applications identified on the faces of the patents-in-suit. These rights were transferred by Mr. Pathak on September 18, 1998. The last significant transfer of any rights in the patents in suit occurred in 2013, the same year where some rights in the patents-in-suit were effectively transferred to plaintiffs Integra LifeSciences Corp. and Integra LifeSciences Sales LLC via the Stock Purchase Agreement of Covidien Group S.A.R.L. by Integra Life Sciences Corporation. Plaintiffs further respond that that they have produced non-privileged documents pursuant to Federal Rule of Civil Procedure 33(d) for which the burden of ascertaining the above requested information is substantially the same for HyperBranch as it is for Plaintiffs. These documents include, for example, the documents identified in Plaintiffs Objections and Response to HyperBranch Interrogatory No. 5 (and supplemental responses thereto) along with the following documents: INT00294034-54, INT00650909-18, INT00651004-05, INT00704790-805, INT00637241-91, INT00477543-93, INT00289244-46, INT00481381-504, INT00289335-42, INT00283427-29, INT00289347-68, INT00289426-46, INT00284501-08, INT00289402-25, INT00704658-723, INT00704724-89, INT00635834-INT00636011, INT00294242-61, and INT00635902-61. Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery in accordance with the Rules

INTERROGATORY NO. 5 [13]. Describe in detail the amount, method of calculation, and all facts and evidence supporting any calculation for any damages You claim in this Action, and specifically identify and explain the damages suffered by each particular Plaintiff.

OBJECTION AND ANSWER TO INTERROGATORY NO. 5 [13]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be a single interrogatory as it contains multiple and distinct subparts. Plaintiffs further object to this interrogatory to the extent it purports to be HyperBranch's fifth interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's thirteenth interrogatory. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs also object to this interrogatory to the extent it constitutes a contention interrogatory that is overly broad, unduly burdensome, and premature at this stage of discovery. Plaintiff further object to this Interrogatory as seeking information that is properly the subject of expert discovery and expert testimony in advance of the schedule set for the disclosure of expert reports and expert discovery as set forth in the Scheduling Order entered by the Court. Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery in accordance with the Rules.

Subject to and without waiver of the foregoing objections and general objections, Plaintiffs respond that such computations cannot be completed until full and complete information is obtained from Defendant. In this case, damages cannot be computed by providing a monetary number as Plaintiffs damages includes aspects for which monetary damages are insufficient to account for the losses due to Defendant's infringing activity. For infringement in

the United States, the monetary damages that only encompass a small portion of the total harm suffered by plaintiffs and would be equal to at least plaintiff's lost profits (or no less than a reasonable royalty) and damages outside of the United States are not less than a reasonable royalty in accordance with 35 U.S.C. §284. Plaintiffs also believe that discovery will establish that this is a case of willful infringement due at least in part to Defendant's receiving notice of infringement in January 2015 and defendant willfully disregarding that notice coupled with Defendant's continuing and increasing infringement after receiving notice of infringement. At least Defendant's willful infringement makes this an exceptional case which warrants Plaintiffs to recover up to 3 times their actual damages and their attorneys fees along with pre and post judgment interest and costs. Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery in accordance with the Rules.

INTERROGATORY NO. 6 [14]. Describe the complete factual and legal basis for Your assertions that any alleged infringement by Defendant is willful.

OBJECTION AND ANSWER TO INTERROGATORY NO. 6 [14]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be HyperBranch's sixth interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's fourteenth interrogatory. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to this Interrogatory as it constitutes a premature contention interrogatory that is overbroad and unduly burdensome and premature at this stage of

the litigation in that it requests identification of "the complete factual and legal basis for Your assertions that any alleged infringement is willful." Subject to and without waiver of the foregoing specific and general objections, Plaintiffs respond that discovery will establish that this is a case of willful infringement due at least in part to Defendant's receiving notice of infringement in January 2015 and defendant willfully disregarding that notice coupled with Defendant's continuing and increasing infringement after receiving notice of infringement. Plaintiffs further incorporate by reference their response to Interrogatory No. 13 as if fully recited herein. Investigation of the facts is ongoing and Plaintiffs reserve the right to supplement this response to identify additional information and/or documents as more facts arise in discovery in accordance with the Rules.

INTERROGATORY NO. 7 [15]. Describe the complete factual and legal basis for Your assertion that this is an exceptional case under 35 U.S.C. § 285.

OBJECTION AND ANSWER TO INTERROGATORY NO. 7 [15]:

Plaintiffs incorporate their General Objections and Objections to Specific Definitions by reference. Plaintiffs object to this interrogatory to the extent it purports to be HyperBranch's seventh interrogatory. HyperBranch previously served Interrogatory Nos. 1-6 on October 23, 2015, and Interrogatory Nos. 7-8 on December 9, 2015. Thus, this interrogatory is HyperBranch's fifteenth interrogatory. Plaintiffs further object to this interrogatory to the extent it seeks the disclosure of information protected by the attorney-client privilege, attorney work-product doctrine, common interest privilege, or any other applicable privilege or protection, as provided by any applicable law. Plaintiffs further object to the interrogatory as overbroad and unduly burdensome and premature at this stage of the litigation in that it requests identification of "the complete factual and legal basis for Your assertion." Plaintiffs respond that this is an exceptional case at least because Defendants infringement has been willful and incorporate their

response to Interrogatory No. 13 as if set forth herein. Investigation of the facts is ongoing and Plaintiffs will supplement this response to identify additional information and/or documents as more facts arise in discovery in accordance with the Rules.

AS TO OBJECTIONS ONLY:

DATED: October 27, 2016

/s/ Karen L. Pascale

An Attorney for Plaintiffs, Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical, Inc., and Incept LLC

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CERTIFICATE OF SERVICE

I, Karen L. Pascale, Esquire, hereby certify that on October 27, 2016, I caused true and correct copies of the foregoing document to be served upon the following counsel of record by e-mail:

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EXHIBIT 8

FULLY REDACTED

Exhibit 9

UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE

Integra LifeSciences Corp., Integra LifeSciences Sales LLC, Confluent Surgical, Inc. and Incept LLC Plaintiffs, v. Hyperbranch Medical Tech., Inc. Defendant.	Civil Action No. 15-819 (LPS) (CJB) Jury Trial Demanded

REBUTTAL EXPERT REPORT OF DR. DENNIS J. RIVET

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I. INTRODUCTION

A. Qualifications and Experience

1. I, Dr. Dennis J. Rivet, II, am an Associate Professor, Department of Neurosurgery at Virginia Commonwealth University in Richmond, VA and an Assistant Professor, Uniformed Services University of the Health Sciences, Bethesda MD. I have previously provided an opening expert report in this case, dated September 8, 2017, which includes a more detailed description of my qualifications, experience (and a copy of my current CV) and opinions as of that date which are incorporated herein by reference.

2. Since the time of my previous expert report the matters in which I have testified or been deposed has not changed. Additionally, I am being compensated at my standard consulting rate per hour for time spent on this matter (which has not changed since the rate identified in my opening report), which is in no way dependent on the outcome of this case.

B. Scope of Matters Addressed

3. I have been asked by Plaintiffs to review the Opening Expert Report of Jonathan Flombaum, Ph.D. Regarding U.S. Patent Nos. 7,009,034, 7,592,418, and 7,532,566 and to give my technical analysis and opinion to that report including my opinions on what Dr. Flombaum characterized as the "predetermined thickness" claims (i.e., claim 20 of the '034 Patent, claims 8 and 23 of the '418 Patent, and claims 4 and 22 of the '566 patent).

4. I have also been asked to review certain portions of Dr. Lowman's Opening Expert Report directed to his assertions of anticipation of claims 1, 6, 7, 12, 17 of U.S. Patent No. 8,535,705 by U.S. Patent No. 6,312,725 to Wallace along with U.S. Patent No. 8,535,705 and U.S. Patent No. 6,312,725 and provide my technical analysis and opinion regarding those opinions from Dr. Lowman. In particular, I have been asked to provide my opinion as to

whether hydrogels of Wallace '725 would have been considered biocompatible in view of the biocompatibility studies of Wallace '725.

5. I have also been asked to address the issue of whether barium sulfate can be used to make a hydrogel that is suitable to coat a tissue of a patient as required by US Patent No. 7,009,034.

II. INFORMATION CONSIDERED IN FORMING MY OPINIONS

6. In forming the opinions expressed in this report, I reviewed and considered all of the materials identified in my opening expert report and my declaration, as well as the report of Jonathan Flombaum and its exhibits along with a portion of Dr. Lowman's report (particularly pp. 319-339) and U.S. Patent Nos. 5,874,500, 6,312,725, 7,000,034 and 8,535,705.

7. I have reviewed videos showing hydrogels containing barium sulfate and fibrillar collagen being applied to a substrate. I have also reviewed video showing hydrogels being removed from the substrate. I understand that the videos are experiments conducted by or at the direction of Dr. Mays.

8. The materials that I reviewed and considered with respect to this rebuttal report are expressly identified throughout the body of this report.

III. SUMMARY OF OPINIONS

9. When using the Court's claim constructions, I disagree with Dr. Flombaum's opinion that, a human being (including a neurosurgeon) is not capable of identifying a color that is correlated to a particular thickness when using the commercial products that practice the inventions claimed in the '034, '418, and '566 patents.

10. The specific examples of the "tetra-SG-PEG+tetra-amino-PEG hydrogel compositions" in U.S. Patent 6,312,725 to Wallace cited by Dr. Lowman as his sole basis for

finding anticipation of the asserted claims of U.S. Patent No. 8,535,705 are not a biocompatible hydrogel as required by the asserted claims of the '5,705 Patent.

11. The hydrogels disclosed in U.S. Patent No. 5,874,500 to Rhee that I understand to have been cited by Dr. Lowman as his basis for his opinions on anticipation and obviousness of the asserted claims of U.S. Patent No. 7,00,034 are not biocompatible and thus not suitable to coat the tissue of a patient as required by the asserted claims of the '034 Patent.

12. A hydrogel containing barium sulfate is neither biocompatible nor suitable for coating a tissue of a patient.

13. A hydrogel containing fibrillar collagen is not suitable for coating a tissue of a patient, and fibrallar collagen does not provide a means for visualizing the hydrogel coating on a patient.

IV. OPINIONS

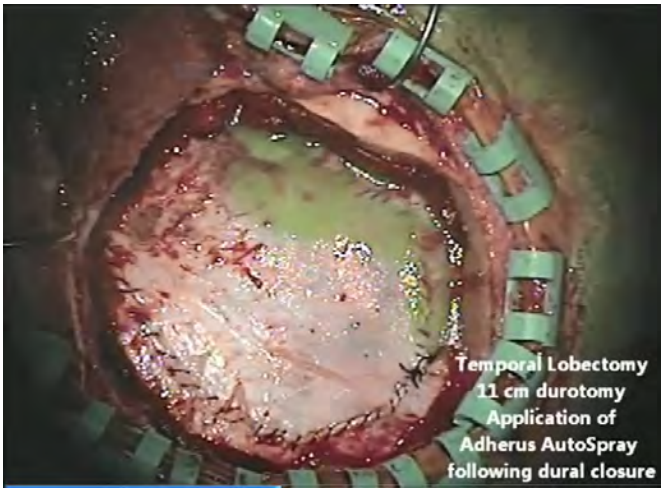
A. Rebuttal to Dr. Flombaum's Opinion

14. As stated in my opening report, it is my opinion based on my years of experience and professional difficulties encountered with earlier products (i.e., fibrin glue) that did not have a visualization agent that provides a visually observable change to gauge a predetermined thickness of the hydrogel, my years of experience with the DuraSeal product, my review of two videos from HyperBranch entitled "Adherus AutoSpray Following Temporal Lobectomy" and "Adherus AutoSpray Preparation" and discussions with my colleagues at VCU who have used the Adherus products that neurosurgeons using either the DuraSeal or Adherus products can and do use the visually observable change caused by the visualization agent in those products to determine whether a predetermined thickness (e.g., 1-2 mm as stated in the instructions for use of

the Adherus products) for the hydrogel coating has been achieved when the Court's July 27, 2017 ruling as to the meaning of those terms is properly applied.

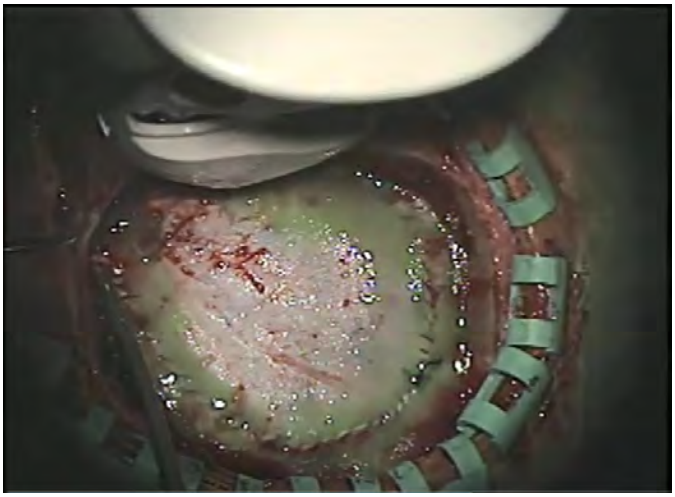
15. As particularly noted in my opening report that based on my own observations, the instructions for use of the Adherus products and the HyperBranch videos "demonstrate that the user understands to stop applying the Adherus product at point when there is a uniform, even coating changing the color of the target site from the natural tissue color to an even green color, and at this point the coating obscures the sutures, subjacent tissue plane or microvasculature indicating the user has reached the predetermined thickness of 1-2 mm specified in the Adherus products IFUs."

16. For example and as seen in the still image below (taken from the HyperBranch video entitled "Adherus AutoSpray Following Temporal Lobectomy") which is after the application of the first pass of the Adherus product, there is not a uniform green color of the Adherus product that allows a user to gauge whether the predetermined thickness (for example 1-2 mm) has been applied.

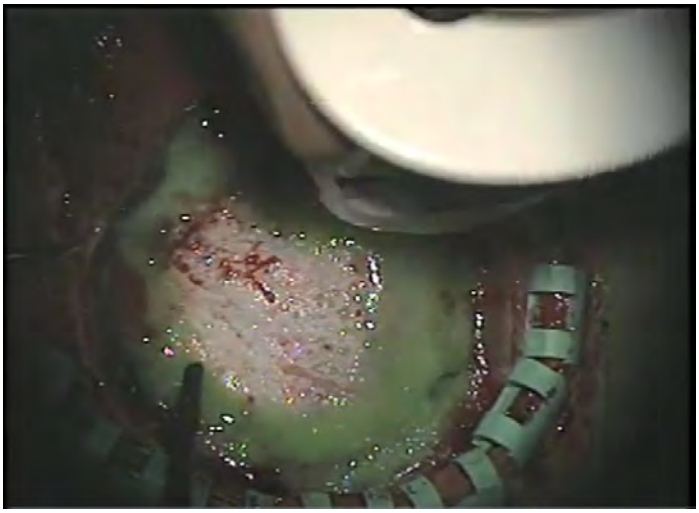


As seen in the still image below (taken from the same HyperBranch video) which is after the second pass of using the Adherus product, there still is not a uniform green color of the Adherus

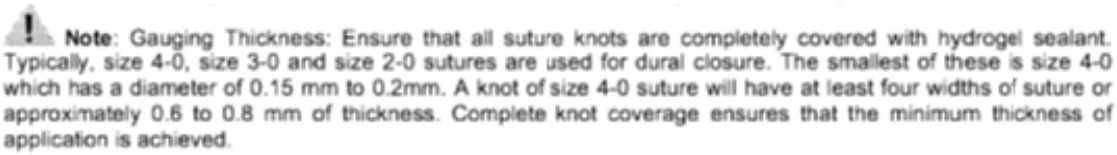
product that allows a user to gauge whether the predetermined thickness (for example, 1-2 mm) has been applied.



Lastly, and as seen in the still image below (taken from the same HyperBranch video), which is after the third pass of using the Adherus product, a uniform green color of the Adherus product is present that at this point obscures/covers the sutures, subjacent tissue plane or microvasculature indicating the user has reached a predetermined thickness (for example 1-2 mm) specified in the Adherus product IFUs



This is especially true if the user has accurately followed the IFUs for the Adherus product as the IFUs particularly describe


Note: Gauging Thickness: Ensure that all suture knots are completely covered with hydrogel sealant. Typically, size 4-0, size 3-0 and size 2-0 sutures are used for dural closure. The smallest of these is size 4-0 which has a diameter of 0.15 mm to 0.2mm. A knot of size 4-0 suture will have at least four widths of suture or approximately 0.6 to 0.8 mm of thickness. Complete knot coverage ensures that the minimum thickness of application is achieved.

17. It is my further opinion that Dr. Flombaum's opinion is based on an inaccurate or incomplete understanding of the Court's relevant claim constructions. Particularly Dr. Flombaum notes the following items throughout his report in describing the requirements of the claims:

- "In my opinion, a human being is not capable of performing a **precise color or transparency matching from memory**, which I understand is a step required by all of the Evaluated Claims" – Paragraph 12 (emphasis added)

- "I understand from Dr. Lowman that the Evaluated Claims require a user to observe the color, color intensity, or transparency of a hydrogel material at a given thickness is a 'test' application and then at some indeterminate time thereafter, **match the same** color, color intensity or transparency from memory…" – Paragraph 19 (emphasis added)

- "…I understand the claims require the individual to be able to **reliably and consistently match the same color, color intensity, or transparency and discriminate from memory** the color, color intensity, or transparency from that observed when the hydrogel is not at the thickness observed in the 'test' application" – Paragraph 19 (emphasis added)

- "… the claims require a user to be able to observe and discriminate in real-time from memory colors, color intensities, or transparencies as the thickness of the deposited material increases and know, from memory, when the 'test' color, color intensity or transparency has been observed" – Paragraph 20

- "…[i]n these experiments, a test subject is shown a color patch, asked to remember it, and then asked to **re-identify the color to the best of his ability from a color wheel, or from a subset of several similar options**. This standard paradigm **parallels the Evaluated Claims requirements** at issue here – i.e.,

whether a user could recognize that **currently applied material matches the color of a previously viewed sample** from memory" – Paragraph 25 (emphasis added)

- "…the research results demonstrate that the **precise color matching** from memory as apparently contemplated and required by the Evaluated Claims is not possible due to inaccuracies, biases and imprecisions inherent to human color memory" – Paragraph 26 (emphasis added)

- "Dr. Lowman has informed me, the Evaluated Claims require a **precise and distinctive color (or transparency) change that correlates with a distinct thickness** of the deposited material" – Paragraph 27 (emphasis added)

- "…a user would be **required** to **match a specific and distinct color** (or intensity) **from memory**…" – Paragraph 28 (emphasis added)

- "…an apt analogy for the color memory-match required by the Evaluated Claims is **choosing a paint color at the store intended to match one already on a wall** …**one is very unlikely to accurately pick out <u>the exact same color</u>** when faced with the many closely related exemplars at the store…" – Paragraph 28 (emphasis added)

18. Nowhere in Dr. Flombaum's report is there an acknowledgement or understanding of the Court's construction that found that the "predetermined thickness was not limited to a single thickness and instead may encompass a range of thickness" thus dispelling the argument that I understand was raised by Defendant that "correlation" required a particular specific color at each particular thickness (i.e., a particular RBG value for every particular thickness). Rather, Dr. Flombaum's opinion that repeatedly calls for "matching" "the exact same color" appears to be based on this same claim construction argument that I understand was raised by HyperBranch and not adopted by the Court. Particularly, I find the analogy of choosing a paint color to match one already on a wall used by Dr. Flombaum to be irrelevant to these patents and a clear indication that Dr. Flombaum's basis for his opinion is inconsistent with the Court's claim constructions in the report and recommendation. My significant personal experience as a neurosurgeon using DuraSeal products and my discussions with my colleagues

who have used the Adherus products confirm that neurosurgeons using the products can see an observable change in the visualization agent and neurosurgeons use that observable change to determine whether they have applied the particular thickness of the product that they previously had determined that they wanted to apply.

B. Examples 4 and 5 of U.S. 6,312,725 to Wallace do not teach a biocompatible hydrogel

19. I understand from the portions of Dr. Lowman's report that I reviewed, that he is specifically relying on Example 4 (Table 4) and Example 5 (Table 5) and specifically the "tetra-SG-PEG+tetra-amino-PEG hydrogel compositions" therein as the support in the '725 Patent to teach all of the limitations of the claims of US 8,535,705 which require that the first precursor, the second precursor and the hydrogel formed therefrom are biocompatible. I understand that claim 1 requires that the hydrogel treat a medical condition of a patient and that claims 6 and 7 call out tissue sealing and tissue coating, respectively. It is my opinion that this disclosure of "tetra-SG PEG + tetra-amino PEG" in Ex. 4 and 5 of the Wallace '725 patent does not disclose a biocompatible hydrogel to treat a medical condition of a patient as required in Claims 1, 6, 7, 12, and 17 of the '5,705 patent.

20. The overreaching requirement in the claims of the '5,705 patent of having a biocompatible hydrogel (as well as biocompatible first and second precursors) is well understood by physicians in general and neurosurgeons in particular when evaluating hydrogel sealants that could be used in neurosurgical applications. As the intended users of products and the actual persons who are practicing the asserted claims of the '5,705 Patent, neurosurgeons (at what I understand to be the time frame encompassing the time of invention of the inventions claimed in the '5,705 patent) would understand that the '5,705 patent provides the following explanation on how to determine whether something is "biocompatible"

> In vivo biocompatibility and degradation life times was assessed by injecting or forming a gelling formulation directly into the peritoneal 45 cavity of a rat or rabbit and observing its degradation over a period of 2 days to 12 months. Alternatively, the degradation was also assessed by prefabricating a sterile implant, made by a process like solution casting, then surgically implanting the implant within an animal body. The degradation of the implant over time was monitored gravimetrically or by chemical analysis. **The biocompatibility of the implant was assessed by standard histological techniques**.

See, '5,705 Patent, Col. 21, ll. 43-54. (emphasis added)

21. The Wallace '725 patent defines biocompatible as "the ability of the compositions of the present invention to be applied to tissues without eliciting significant inflammation and fibrosis or other adverse tissue responses." See, '725 Patent, Col. 3, ll. 64-67. In Table 3 (reproduced below) the Wallace '725 Patent also describes a standard histological assessment for its hydrogels through the "Grading Key for Biocompatibility Experiments" done in the '725 patent.

TABLE 3

Grading Key for Biocompatibility Experiments

Score	Gross Observations	Histological Observations
-	all tissues appeared normal	all tissues appeared normal, no inflammation
+	mild foreign body response	mild inflammation
++	moderate foreign body response	moderate inflammation
+++	marked foreign body response	marked inflammation
++++	severe foreign body response	severe inflammation

22. Using the key in Table 3 it is clear that the "Results for Biocompatibility Experiments" in Table 4 of the '725 patent, for the results "tetra-SG-PEG+tetra-amino-PEG hydrogel compositions" formed on tissue (Test C) cited by Dr. Lowman have "severe foreign body response" and "severe inflammation" or as further described by the Wallace '725 patent

"Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation." See, '725 Patent, Col. 13, ll. 37-41.

TABLE 4

Results for Biocompatibility Experiments

Test	Description	Results: Gross Observations	Results: Histological Observations
A	surgical control	–	+
B	fibrillar collagen	–	+
C	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000	++++	++++
D	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-sulfhydryl PEG 10,000	++	++
E	20% w/v tetra-SG PEG 10,000 + 20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000	+	++
F	20% w/v tetra-SG PEG 10,000 + 20% w/v di-sulfhydryl PEG 3,400; gelled ex-vivo; treated with di-amino PEG 3400	++++	++++

23. In my opinion, the result showing "severe foreign body response" and "severe inflammation" would indicate that the hydrogel used in those experiments was not "biocompatible" according to the Wallace '725 Patent. Hydrogels are used to coat or seal tissues of a patient as a medical treatment, such as an adjunct to surgery. Physicians (especially neurosurgeons) would have wanted to avoid a severe foreign body response and severe inflammation in response to a hydrogel at a surgical site that Wallace associates with hydrogels made with amino-PEG. Further, the thick encapsulation of the hydrogel and abscess formation reported for the hydrogel would also indicate that the hydrogel is not biocompatible. The reported results for this hydrogel are in opposite to Wallace's own definition of a biocompatible hydrogel as one that can "be applied to tissues without eliciting significant inflammation and

fibrosis or other adverse tissue responses." In my opinion, this hydrogel of Wallace '725 is not biocompatible by Wallace's own definition.

C. The Hydrogels Disclosed in U.S. Patent 5,874,500 to Rhee Do Not Teach a Biocompatible Hydrogel

24. I understand that Dr. Lowman contends that hydrogels disclosed in U.S. 5,874,500 to Rhee anticipate the asserted claims of the '034 patent and render those claims obvious.

25. I have reviewed U.S. Patent 5,874,500 to Rhee and I understand that it discloses hydrogels formed using a first precursor with electrophilic functional groups and a second precursor with nucleophilic functional groups (amino and thiol functional groups).

26. I have compared the disclosure of the Rhee '500 patent to the disclosure of the Wallace '725 patent. Particularly, I reviewed Tables 3 and 4 of the Wallace '725 patent, to see if the biocompatibility experiments therein were of assistance in determining whether the hydrogels of the Rhee '500 patent were biocompatible. As discussed above with respect to the Wallace '725 patent, Test C in the Wallace '725 patent involved hydrogels formed using "amino-PEG" (an amino functional group) as the second precursor. Test D in the Wallace '725 patent involved hydrogels using a second precursor with thiol functional groups (i.e., "tetra-sulfhydral PEG"). As discussed above, the Test C hydrogels elicited "severe foreign body response" and "severe inflammation." The Test D hydrogels elicited "marked moderate foreign body response" and "severe moderate inflammation." A neurosurgeon, presented with such testing, would characterize the hydrogels as not being biocompatible and not want to use them in a medical procedure. Further it is my opinion that a neurosurgeon, would understand that based on Wallace's biocompatibility testing and an understanding as to the similarity of the hydrogels of Rhee (also formed using amino and thiol groups as the nucleophilic functional groups) to the

above hydrogels from the Wallace '725 patent that the similar hydrogels of the Rhee '500 patent are also not biocompatible.

D. Hydrogels Containing Barium Sulfate Are Neither Biocompatible Nor Suitable to Coat Tissue of a Patient

27. Neurosurgeons, like most physicians, are aware of the general uses for barium sulfate and the limitations of using barium sulfate in the body. For example, barium sulfate is typically used in the medical field as a radiocontrast agent in X-rays and other radiographic studies of the gastrointestinal (GI) tract because the GI tract is the only part of the human body having tissue from which barium sulfate can be readily removed. See "Contrast Media in Roentgen Diagnosis", p. 614 (copy attached). It is widely known that barium sulfate causes an inflammatory response within days after contact with human tissue and its toxicity to human tissue has been widely studied. See e.g., "The Structure of Mononuclear Phagocyte Differentiating in Vivo" p. 340 (noting that barium sulfate is known to cause "granulomas resembling those classically described as foreign-body lesions" and causes significant inflammation within a short amount of time after contacting with tissue)(copy attached). The study discussed in "The Structure of Mononuclear Phagocyte Differentiating in Vivo", for example, describes the following timeline of reactions after tissue is contacted with barium sulfate:

- After 3 days -- "[a] large central core of uningested barium is surrounded by a thin rim of small mononuclear cells which loosely infiltrate the surrounding tissues . . . ingestion of the barium is minimal." p. 336.
- After 7 days – a "dense sheet of mononuclear phagocytes" with "[a]lmost all of the barium . . . is contained within phagocytes. The mononuclear phagocytes are predominantly mature macrophages which have definite cytoplasmic borders and form small, early foreign-body granulomas." p. 336-337.
- After 10 days – "[w]ell-formed foreign-body granulomas constitute the response to barium [sulfate]. The macrophages are densely packed, and their abundant

cytoplasm is distended with phagocytosed barium. The closely aggregated, mature macrophages form solid sheets and nests." p. 337.

- Between 15-25 days – "[t]he granulomas are essentially unchanged, but more giant cells are present." p. 337.

As seen below in excerpts from additional studies, the same toxic effects of barium sulfate exposure to tissue are found:

Huston and co-workers[3] injected barium sulfate suspensions (80 to 90 per cent) endotracheally into rats, and the animals were sacrificed at intervals varying from 30 minutes to 72 hours; and from 7 to 126 days. At 12 to 24 hours there was an acute inflammatory reaction. From 48 hours to 15 days there was mononuclear infiltration associated with areas of consolidation containing barium. The mononuclear cells were packed with particles of barium. During the next 15 to 30 days the mononuclear cells disintegrated and the barium salt was set free. Tissues studied at 94 to 120 days revealed an occasional small solid area related to the presence of refractile masses with occasional lymphocytes, monocytes, and giant cells. There was no fibrosis.

It is evidently not correct to regard these lesions as strictly barium sulfate granulomas. In no instance was the reaction caused by barium sulfate alone. The conditions under which these granulomas occurred include the escape of either gastric or fecal contents. These two components certainly elicited severe inflammatory reactions. However, the barium sulfate did contribute to the inflammatory reaction by superimposing the presence of both extra- and intracellular anisotropic crystals, macrophages, and giant cells. The lymphocytes, plasma cells, monocytes, and fibroblasts presented 1 part of the complex inflammatory reaction. No organized granulomatous structures have been described nor were any seen in our patient. There is little doubt that the fecal components contribute to the tissue reaction.

SUMMARY

A patient with acute appendicitis was given a barium enema and 31 days later the appendix was removed. A granulomatous reaction to the barium sulfate was found in and about the appendix. This had formed apparently as a

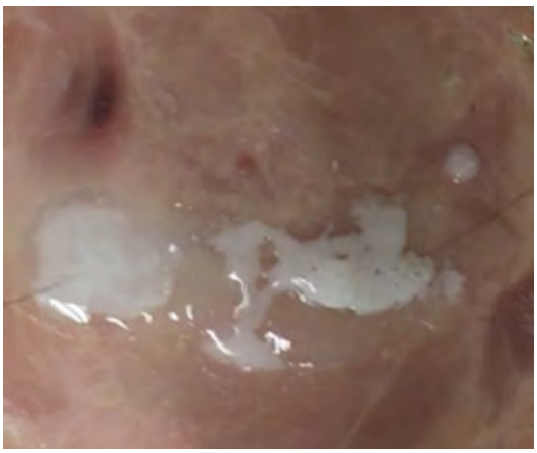
"Granulomatous Reaction to Barium Sulfate in and about Appendix", p. 159 (copy attached) and

Summary: Particulate polymethylmethacrylate debris has been implicated in the inflammatory response observed surrounding loosened cemented implants. The rat subcutaneous pouch model and the Howie implant model (used to study bone resorption) were used to quantify the response to mechanically produced endotoxin-free polymethylmethacrylate debris with and without 10% (wt/vol) $BaSO_4$. In the rat subcutaneous pouch model, the inflammatory response to polymethylmethacrylate particles containing $BaSO_4$ was greater than the response to plain polymethylmethacrylate particles of similar size. Increased inflammation was measured by leukocyte counts and levels of prostaglandin E_2, tumor necrosis factor, and neutral metalloprotease. In addition, particulate polymethylmethacrylate with $BaSO_4$ caused significantly greater bone resorption in the Howie model than did particulate plain polymethylmethacrylate. In *in vitro* studies, particulate polymethylmethacrylate with $BaSO_4$ stimulated more prostaglandin E_2, neutral metalloprotease, and tumor necrosis factor from human monocytes in culture and stimulated greater proliferation of synovial cells than did particulate plain polymethylmethacrylate. The presence of $BaSO_4$ appears to significantly intensify the inflammatory response to polymethylmethacrylate debris.

"Comparison of the Inflammatory Response", p. 532 (copy attached).

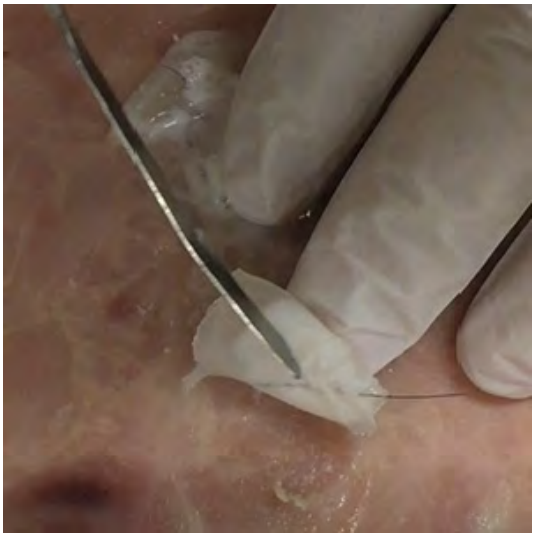
28. The above study is particularly instructive because it involves implants including barium sulfate and how barium sulfate can affect tissue if tissue is exposed to the barium sulfate from the implant. The '034 patent makes clear that the claimed hydrogels are applied to a patient and must be suitable to coat a tissue of a patient such as a tissue coating or tissue sealant. Suitable to coat a tissue of a patient therefore requires at least biocompatibility. I believe that it also requires that the hydrogel have sufficient structural integrity and be sufficiently adherent so as to be suitable to coat a tissue of a patient. Such hydrogels can also be degradable. The '034 patent teaches whether a hydrogel is biocompatible is determined from "assessment by standard histological techniques." See, '034 Patent, col. 29, ll. 43-49.

29. I have reviewed a video having the file name "BaSO4 Visualization with Adherus Dural Sealant.mov" that I understand was created by Dr. Mays whereby barium sulfate was mixed with reactive precursors and applied to a tissue substrate. I understand that the hydrogel was made using 1.5 wt% of barium sulfate added to the amine component of the Adherus Dural Seal kit and a 4-arm PEG with a molecular weight of 5 kg/mol was substituted for the colored Adherus PEG. I further understand the Adherus Dural Sealant applicator was used to apply the hydrogel. A still shot from that video is provided below:



30. I have also reviewed a video having the file name "BaSO4 Visualization with Adherus Dural Sealant Hydrogel Performance.mov" that I understand was created by Dr. Mays showing the removal of the hydrogel from the tissue substrate. As one can see, barium sulfate, being an insoluble powder was not evenly distributed throughout the hydrogel. Instead, it collected at unpredictable locations in the hydrogel in a clumping effect. The remainder of the hydrogel had comparatively little barium sulfate and did not impart color or obscure the optical clarity of the hydrogel. In my opinion, the barium sulfate did not provide a means for visualization of the coating or a suitable visualization agent as a neurosurgeon would want to see that the barium sulfate was evenly distributed at the point of application of the hydrogel and not unpredictably applied in clumps. As shown in the still shot from that video provided below, the hydrogel was easily removed from the surface of the tissue substrate (i.e., had minimal adherence) and appeared to have minimal structural integrity for a tissue coating or tissue sealant compared to the commercial products I am familiar with. I do not know whether the minimal adherence or structural integrity is because of the barium sulfate. However, the collected barium

sulfate looked to prevent any adherence of the hydrogel and it appears to be contacting the tissue directly.



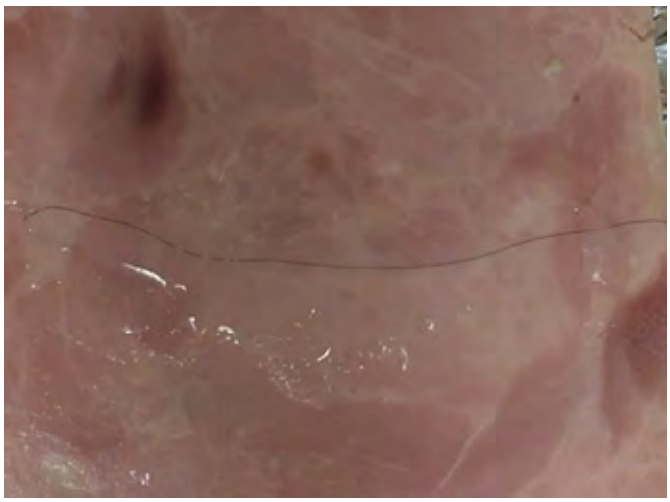
31. A neurosurgeon (an intended user of products and an actual person who would practice the asserted claims of the '034 Patent) would know that the hydrogels encompassed by the claims must be biocompatible and have no toxic effects upon application or degradation within the desired timeframe. A neurosurgeon would consider any hydrogel that is not biocompatible or that has toxic effects upon application or degradation within a desired timeframe to be unsuitable as a composition to coat the tissue of a patient. In view of the known toxic effects that barium sulfate has on tissue after prolonged exposure, the requirement of a degradable hydrogel, the lack of water solubility and resulting poor dispersion within a hydrogel, and the lack of absorption and ready removal from tissue other than in the GI tract of barium sulfate – a neurosurgeon would understand that a hydrogel having barium sulfate would result in the prolonged exposure to barium sulfate by the patient's tissue upon application and after the

hydrogel degrades, would result in the toxic effects of prolonged exposure to barium sulfate. Consequently, a neurosurgeon would consider a hydrogel with barium sulfate to be neither biocompatible nor suitable to coat a tissue of a patient.

32. In addition, given the lack of solubility of barium sulfate in aqueous media and the resulting concentration or collecting of the barium sulfate in the hydrogel and its resulting negative effect, I do not believe that barium sulfate is a visualization agent because it does not result in a useful hydrogel suitable to coat a tissue of a patient and does not impart color or obscure optical clarity to the entirety of the hydrogel. The above-videos I observed showed lack of dispersibility of barium sulfate and lack of controlled application of the hydrogel to a tissue substrate. Where the barium sulfate did not collect, I observed that the barium sulfate did not impart a color change or obscure optical clarity of the hydrogel.

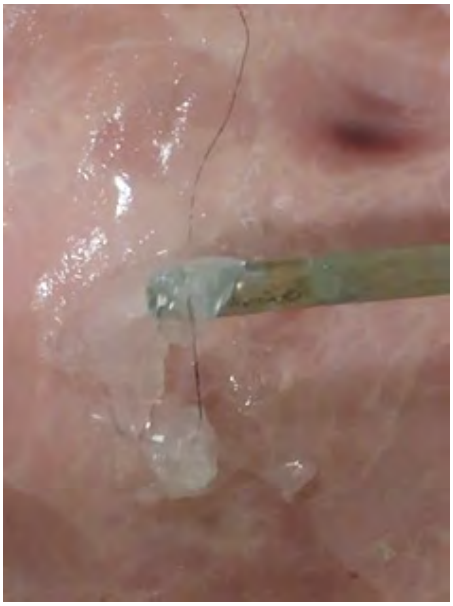
E. Hydrogels Containing Fibrillar Collagen Are Not Suitable to Coat Tissue of a Patient

33. I have also viewed a video of the making of a hydrogel that included fibrillar collagen (file name "Fc Spraying with Adherus Dual Sealant.mov) that I understand was also created by or at the direction of Dr. Mays. It is my understanding that 0.1 wt% fibrillar collagen was added to the amine component of the Adherus Dural Seal kit, and that 4-arm PEG with MW 5 kg/mol was substituted for the colored Adherus PEG. It is my understanding that the hydrogel was applied using the Adherus Dural Sealant applicator. A still shot from that video is provided below:



34. I note that the hydrogel was essentially clear and that the fibrillar collagen neither imparted color not obscured the optical clarity of the hydrogel. It also appeared from the video that the hydrogel with fibrillar collagen was very hard to administer as a result of clogging during application. In other words, the video I observed showed lack of dispersibility of fibrillar collagen and lack of controlled application of the hydrogel to a tissue substrate.

35. I have also reviewed a video having the file name "Fc Spraying with Adherus Dural Sealant-mechanical performance.mov" that I understand was created by or at the direction of Dr. Mays showing the removal of the hydrogel from the tissue substrate. As shown in the video and still shot below, the hydrogel was easily removed from the surface of the tissue substrate (i.e., had minimal adherence) and appeared to have minimal structural integrity for a tissue coating or tissue sealant compared to the commercial products I am familiar with. I do not know whether the minimal adherence or structural integrity is because of the fibrillar collagen.



36. Accordingly, in my opinion, the fibrillar collagen is not a visualization agent and a hydrogel containing fibrillar collagen is not suitable to coat a tissue of patient.

V. TRIAL EXHIBITS

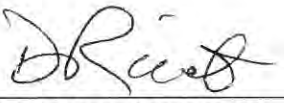
37. I have not determined what trial exhibits or demonstrative exhibits that I might use if I testify in this case, but they will most likely be derived from the materials that I considered in connection with preparing my original report, this report, portions of these reports, and/or exhibits to these reports.

38. Pursuant to Fed. R. Civ. P. 26(e)(1), I reserve the right to supplement this report as appropriate. In addition, I reserve my right to modify this report to the extent that the Court adopts a claim construction for any above-identified claim limitation that is inconsistent or different from what I noted above.

VI. CONCLUSION

39. In conclusion, it is my opinion that a neurosurgeon using either the DuraSeal or Adherus products would be able to and does in fact rely on an observable change of the visualization agent in the hydrogel to determine when a predetermined thickness of the hydrogel has been formed. The hydrogels identified by Dr. Lowman in the Wallace '725 patent as the basis for his opinion on anticipation of the asserted claims of the '5,705 patents are not biocompatible as required by the asserted claims of the '5,705 patent. A hydrogel that uses barium sulfate would not be biocompatible or suitable to coat a tissue of a patient. The barium sulfate in the hydrogel on the tissue substrate would not be characterized as a visualization agent. Additionally, a hydrogel that uses fibrillar collagen does not impart color or obscure the optical clarity of the hydrogel and its difficult in application and poor structural integrity and mechanical performance would prevent it from being suitable to coat tissue of a patient.

I hereby declare under penalty of perjury that the foregoing is true and correct. Executed on October 2, 2017.



Dr. DENNIS J. RIVET, II.

Exhibit 10

US007009034B2

(12) **United States Patent**
Pathak et al.

(10) **Patent No.: US 7,009,034 B2**
(45) **Date of Patent: *Mar. 7, 2006**

(54) **BIOCOMPATIBLE CROSSLINKED POLYMERS**

(75) Inventors: **Chandrashekhar P. Pathak**, Austin, TX (US); **Amarpreet S. Sawhney**, Lexington, MA (US); **Peter G. Edelman**, Franklin, MA (US)

(73) Assignee: **Incept, LLC**, Lexington, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 417 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/010,715**

(22) Filed: **Nov. 9, 2001**

(65) **Prior Publication Data**

US 2003/0012734 A1 Jan. 16, 2003

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/147,897, filed as application No. PCT/US97/16897 on Sep. 22, 1997, now abandoned, application No. 10/010,715, which is a continuation-in-part of application No. 09/454,900, filed on Dec. 3, 1999, now Pat. No. 6,566,406.

(60) Provisional application No. 60/110,849, filed on Dec. 4, 1998, provisional application No. 60/040,417, filed on Mar. 13, 1997, provisional application No. 60/039,904, filed on Mar. 4, 1997, provisional application No. 60/026,526, filed on Sep. 23, 1996.

(51) **Int. Cl.**

C09F 15/00	(2006.01)
C07K 1/00	(2006.01)
C08G 63/48	(2006.01)
A61K 47/30	(2006.01)

(52) **U.S. Cl.** **530/200**; 530/350; 530/356; 530/382; 530/402; 525/54.1; 525/54.11; 525/54.2; 424/428; 424/486; 424/488

(58) **Field of Classification Search** 530/200, 530/350, 380, 382; 435/810; 525/54.1, 525/54.11, 54.2; 528/354, 361; 424/428, 424/486, 488

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,520,949	A	7/1970	Shepard et al.	
4,414,976	A	11/1983	Schwarz et al.	128/334
4,427,651	A	1/1984	Stroetmann	424/46
4,601,286	A	7/1986	Kaufman	128/132
4,646,730	A	3/1987	Schonfeld et al.	728/156
4,717,378	A	1/1988	Perrault et al.	604/20
4,925,677	A	5/1990	Feijen	424/484
4,937,270	A	6/1990	Hamilton et al.	514/777
5,104,909	A	4/1992	Grasel et al.	521/159
5,192,743	A *	3/1993	Hsu et al.	514/8
5,213,760	A	5/1993	Dziabo, Jr. et al.	422/37
5,213,808	A	5/1993	Bar-Shalom et al.	424/473
5,281,662	A	1/1994	Ito et al.	525/54.1
5,296,518	A	3/1994	Grasel et al.	521/176
5,410,016	A *	4/1995	Hubbell et al.	528/354
5,423,821	A	6/1995	Pasque	606/74
5,431,639	A	7/1995	Shaw	604/264
5,514,379	A	5/1996	Weissleder et al.	424/426
5,527,856	A	6/1996	Rhee et al.	525/54.1
5,529,914	A	6/1996	Hubbell et al.	435/182
5,550,188	A	8/1996	Rhee et al.	525/54.1
5,573,934	A	11/1996	Hubbell et al.	435/177
5,580,923	A	12/1996	Yeung et al.	525/54.1
5,583,114	A	12/1996	Barrows et al.	514/21
5,605,938	A	2/1997	Roufa et al.	514/59
5,612,052	A	3/1997	Shalaby	424/426
5,626,863	A	5/1997	Hubbell et al.	424/426
5,672,622	A	9/1997	Hedgepeth et al.	514/424
5,681,576	A	10/1997	Henry	424/426
5,702,361	A	12/1997	Evans et al.	604/53
5,744,545	A	4/1998	Rhee et al.	525/54.1
5,752,974	A	5/1998	Rhee et al.	606/214
5,773,025	A	6/1998	Baichwal	424/458
5,776,493	A	7/1998	Barclay et al.	424/468
5,786,421	A	7/1998	Rhee et al.	525/54.1

(Continued)

FOREIGN PATENT DOCUMENTS

EP 0 246 380 A2 10/1986

(Continued)

OTHER PUBLICATIONS

R. Dunn, MD., M. Lyman, B.S., P. Edelman, Ph.D., and P. Campbell, Ph.D., "Evaluation of the SprayGel™ adhesion barrier in the rat cecum abrasion and rabbit uterine horn adhesion models", Fertility and Sterility, vol. 75, No. 2, Feb. 2001, pp. 411-416.

(Continued)

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(57) **ABSTRACT**

Biocompatible crosslinked polymers, and methods for their preparation and use, are disclosed in which the biocompatible crosslinked polymers are formed from water soluble precursors having electrophilic and nucleophilic functional groups capable of reacting and crosslinking in situ. Methods for making the resulting biocompatible crosslinked polymers biodegradable or not are provided, as are methods for controlling the rate of degradation. The crosslinking reactions may be carried out in situ on organs or tissues or outside the body. Applications for such biocompatible crosslinked polymers and their precursors include controlled delivery of drugs, prevention of post-operative adhesions, coating of medical devices such as vascular grafts, wound dressings and surgical sealants. Visualization agents may be included with the crosslinked polymers.

22 Claims, 10 Drawing Sheets

U.S. PATENT DOCUMENTS

5,800,541	A	9/1998	Rhee et al.	623/11
5,801,033	A	9/1998	Hubbell et al.	435/182
5,814,621	A	9/1998	Kanaya et al.	514/54
5,830,178	A	11/1998	Jones et al.	604/49
5,830,196	A	11/1998	Hicks	604/280
5,844,023	A	12/1998	Tomka	524/47
5,869,096	A	2/1999	Barclay et al.	424/468
5,874,500	A *	2/1999	Rhee et al.	525/54.1
5,936,035	A	8/1999	Rhee et al.	525/54.1
5,986,043	A	11/1999	Hubbell et al.	528/354
6,017,301	A	1/2000	Schwartz et al.	547/781
6,020,326	A	2/2000	Roufa et al.	514/59
6,051,248	A	4/2000	Sawhney et al.	424/426
6,124,273	A	9/2000	Drohan et al.	514/55
6,136,333	A	10/2000	Cohn et al.	424/423
6,162,241	A	12/2000	Coury et al.	606/214
6,166,130	A	12/2000	Rhee et al.	525/54.1
6,201,065	B1	3/2001	Pathak et al.	525/90
6,238,403	B1	5/2001	Greene, Jr. et al.	606/108
6,303,102	B1	10/2001	Schlichte	424/10.3
6,312,725	B1	11/2001	Wallace et al.	424/484
6,323,278	B1	11/2001	Rhee et al.	525/54.1
6,465,001	B1	10/2002	Hubbell et al.	435/174
6,566,406	B1 *	5/2003	Pathak et al.	514/772.1

FOREIGN PATENT DOCUMENTS

EP	0 246 380 A3	10/1986
EP	0 414 848	1/1990
EP	0 863 933	11/1996
WO	97/19973	5/1997
WO	97/22371	6/1997
WO	97/22372	6/1997
WO	WO 98/35631	8/1998
WO	99/08718	2/1999
WO	00/09087	2/2000
WO	00/33764	6/2000
WO	01/68155	9/2001

OTHER PUBLICATIONS

R. Ferland, D. Mulani and P.K. Campbell, "Evaluation of a sprayable polyethylene glycol adhesion barrier in a porcine efficacy model", Human Reproduction, vol. 16, No. 12, pp. 2718-2723.

V.R. Jacobs, E. Lehmann-Willenbrock, M. Kiechle, L. Mettler, "SprayGel™ as New Intraperitoneal Adhesion Prevention Method for Use in Laparoscopy and Laparotomy", ISGE 10 Convention, Chicago, Mar. 2001.

V.R. Jacobs, D.A. Melanson, P.K. Campbell, M. Kiechle, A.S. Sawhney, "A Pressure-Balanced Sprayer for Intraabdominal Application of Soluble Biomaterials in Laparoscopy", ISGE 10 Convention, Chicago, Mar. 2001.

R. Ferland, M.D., D. Alan Johns, M.D., P.K. Campbell, Ph.D., A.S. Sawhney, Ph.D., "Evaluation of SprayGel™ Adhesion Barrier System as a Barrier for the Prevention of Adhesion Formation After Gynecological Surgery", ISGE 10, Chicago, Mar. 2001.

US 6,214,374, 04/2001, Schmirler et al. (withdrawn)

* cited by examiner

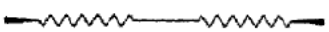

FIG. 1A

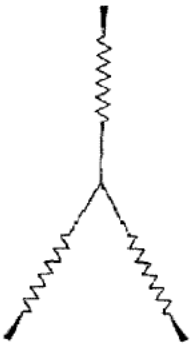

FIG. 1B

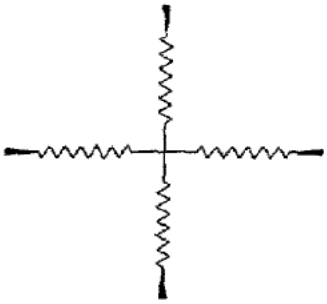

FIG. 1C

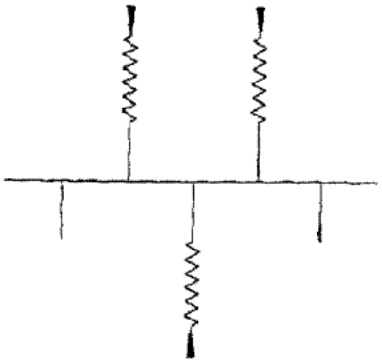

FIG. 1E

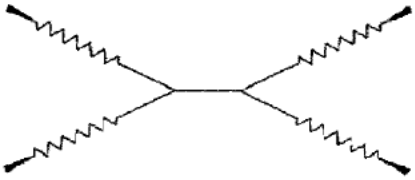

FIG. 1D

FIG. 2F

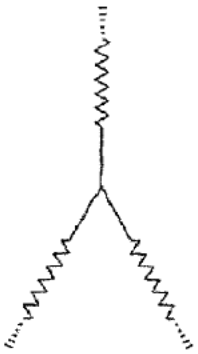

FIG. 2G

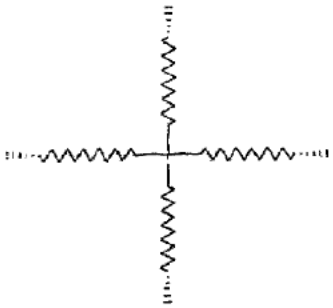

FIG. 2H

FIG. 2J

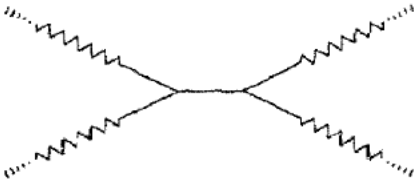

FIG. 2I

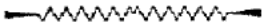

FIG. 3K

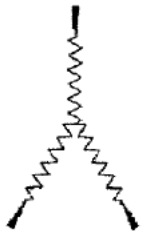

FIG. 3L

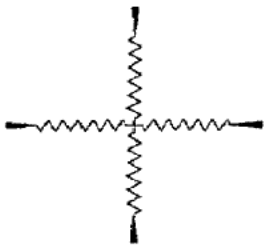

FIG. 3M

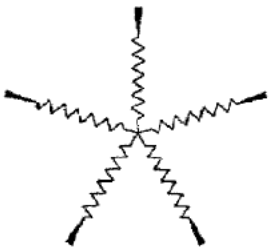

FIG. 3O

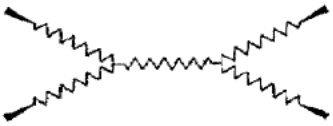

FIG. 3N

FIG. 4Q

FIG. 4P

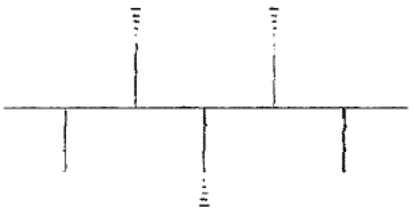

FIG. 4T

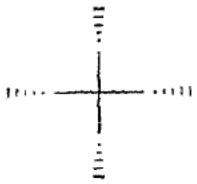

FIG. 4R

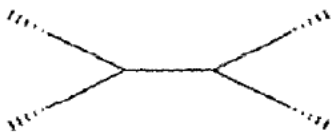

FIG. 4S

FIG. 5U

FIG. 5V

FIG. 5W

FIG. 5Y

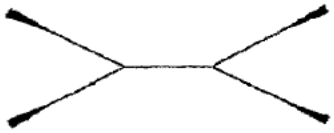

FIG. 5X

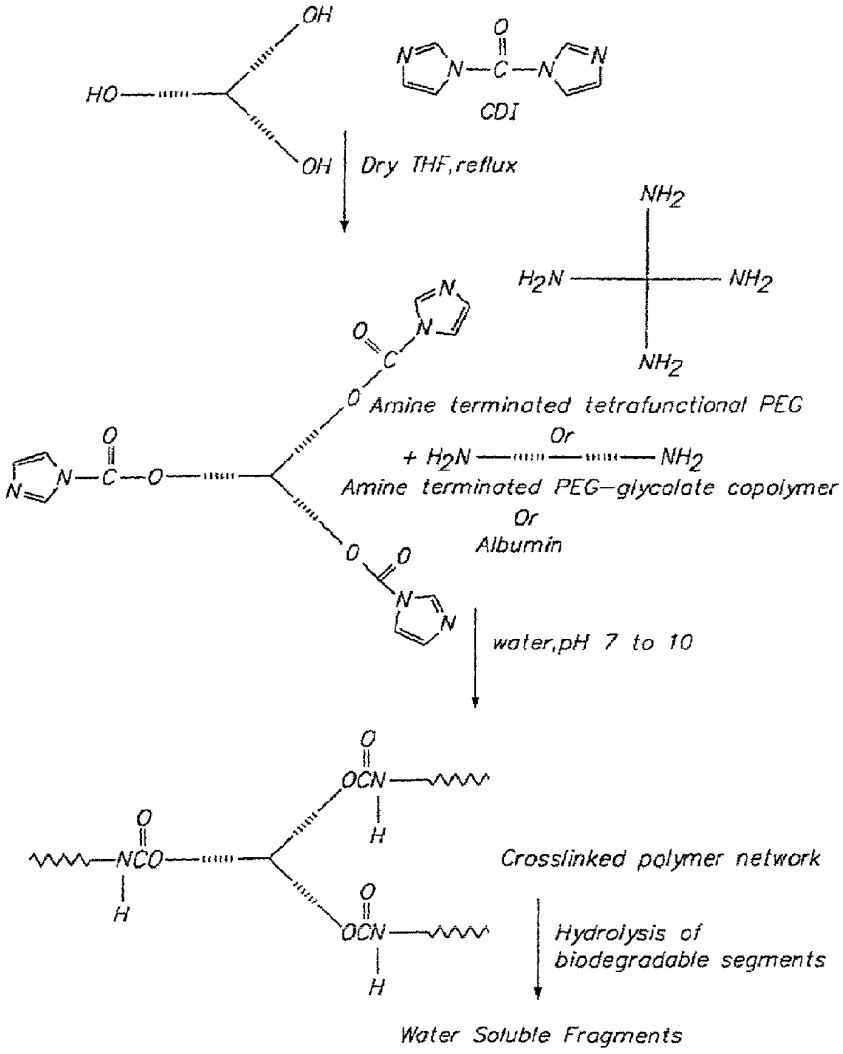
OH
HO
OH
CDI
Dry THF, reflux
NH2
H2N
NH2
NH2
Amine terminated tetrafunctional PEG
Or
+ H2N
NH2
Amine terminated PEG–glycolate copolymer
Or
Albumin
water, pH 7 to 10
OCN
H
NCO
H
OCN
H
Crosslinked polymer network
Hydrolysis of
biodegradable segments
Water Soluble Fragments

FIG. 6

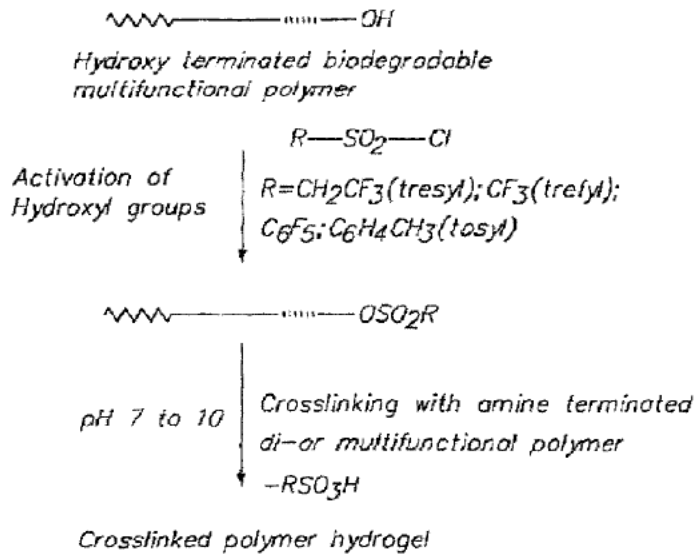
—OH
Hydroxy terminated biodegradable multifunctional polymer
R—SO2—Cl
Activation of Hydroxyl groups
R=CH2CF3(tresyl); CF3(trefyl); C6F5; C6H4CH3(tosyl)
—OSO2R
pH 7 to 10
Crosslinking with amine terminated di-or multifunctional polymer
—RSO3H
Crosslinked polymer hydrogel

FIG. 7

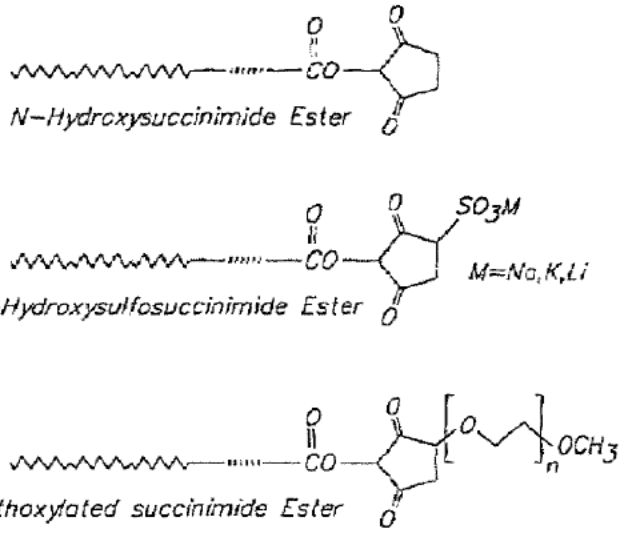
N-Hydroxysuccinimide Ester
SO3M
M=Na, K, Li
N-Hydroxysulfosuccinimide Ester
OCH3
N-Hydroxyethoxylated succinimide Ester

FIG. 9

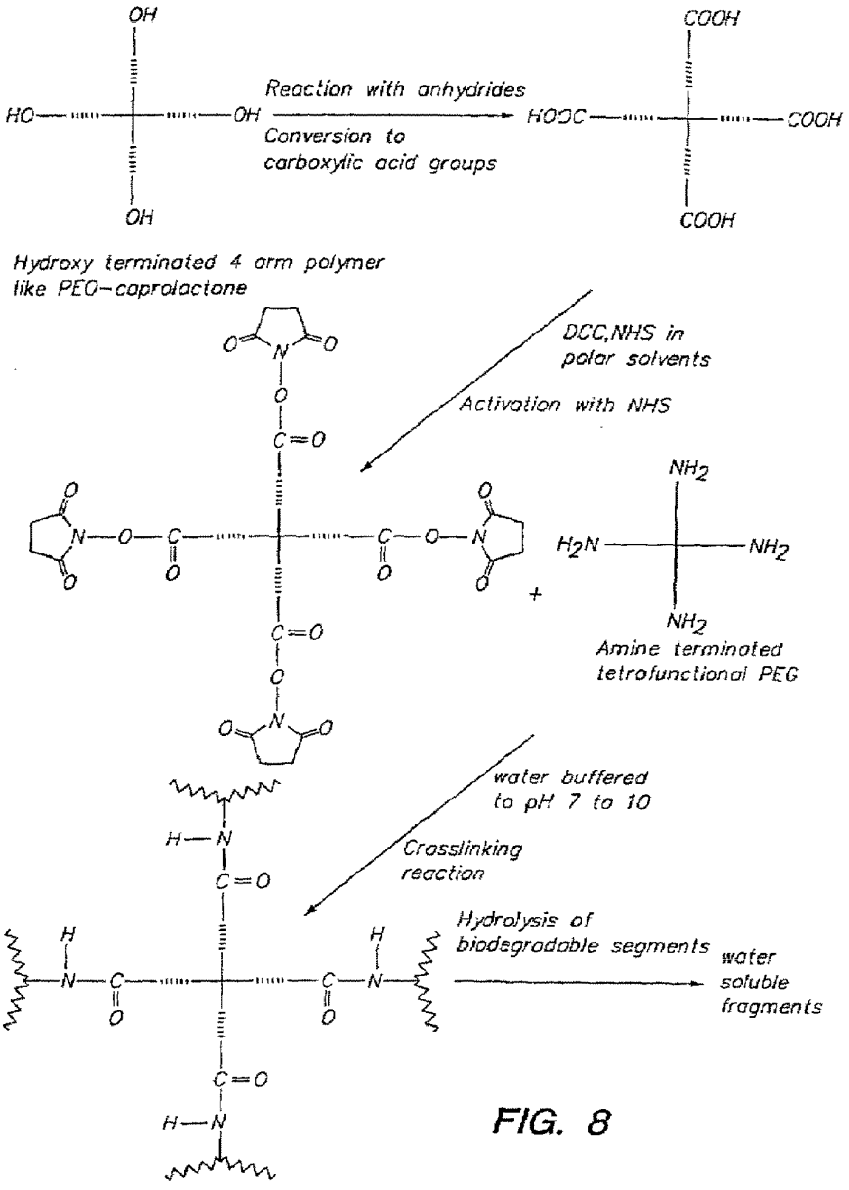
OH
HO
OH
OH
Reaction with anhydrides
Conversion to
carboxylic acid groups
COOH
HOOC
COOH
COOH
Hydroxy terminated 4 arm polymer
like PEG–caprolactone
DCC,NHS in
polar solvents
Activation with NHS
NH2
H2N
NH2
NH2
Amine terminated
tetrofunctional PEG
+
water buffered
to pH 7 to 10
Crosslinking
reaction
Hydrolysis of
biodsgradable segments
water
soluble
fragments

FIG. 8

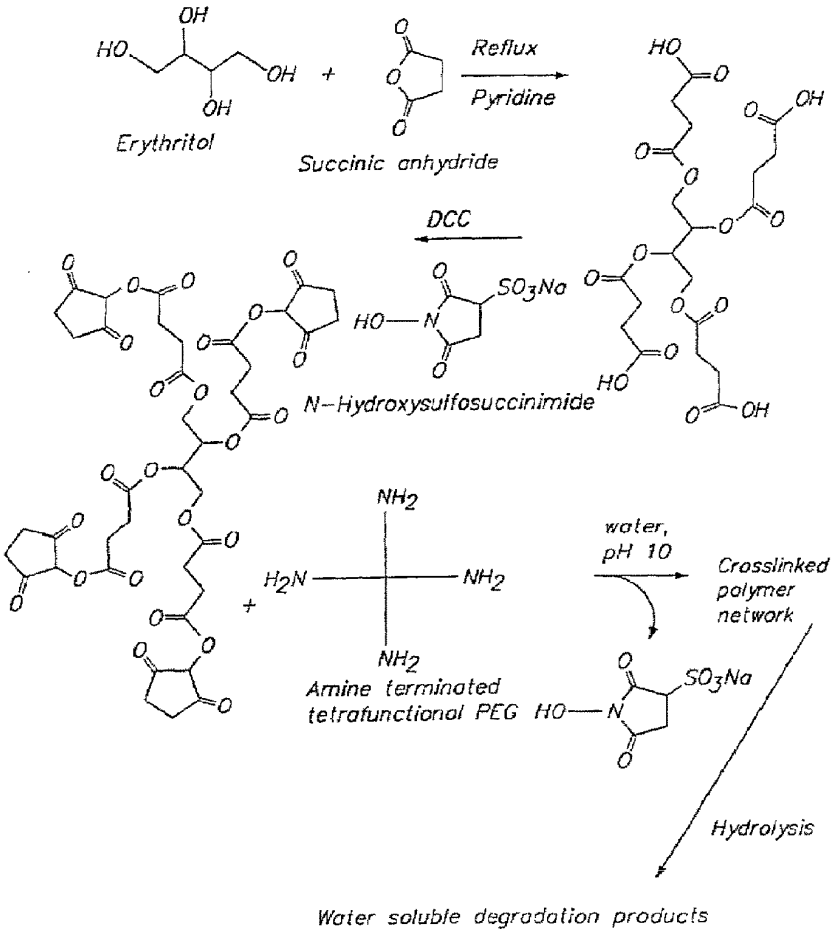
Erythritol
Succinic anhydride
Reflux
Pyridine
DCC
N-Hydroxysulfosuccinimide
Amine terminated tetrafunctional PEG
water, pH 10
Crosslinked polymer network
Hydrolysis
Water soluble degradation products

FIG. 10

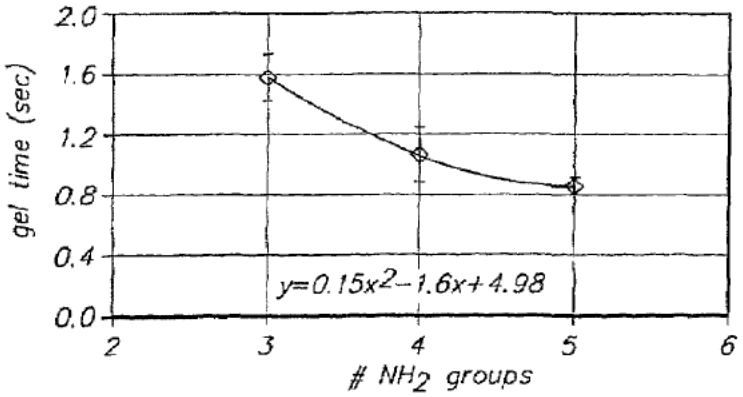
2.0
1.6
1.2
0.8
0.4
0.0
gel time (sec)
y=0.15x2−1.6x+4.98
2
3
4
5
6
NH2 groups

FIG. 11

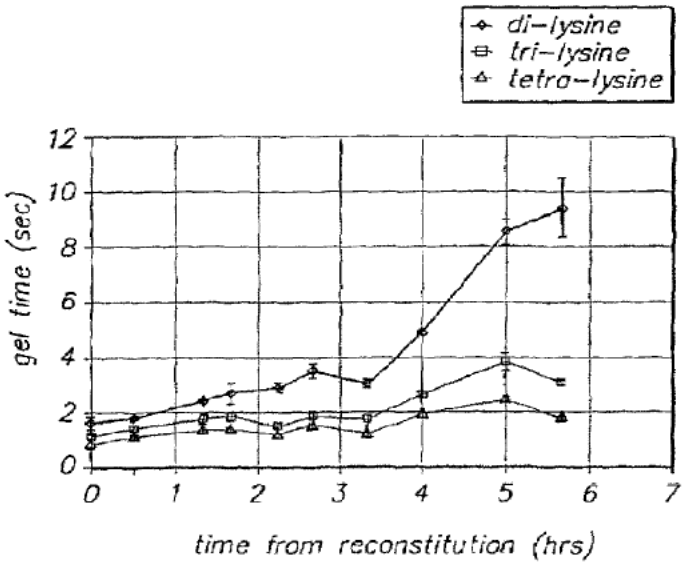
di-lysine
tri-lysine
tetro-lysine
12
10
8
6
4
2
0
gel time (sec)
0
1
2
3
4
5
6
7
time from reconstitution (hrs)

FIG. 12

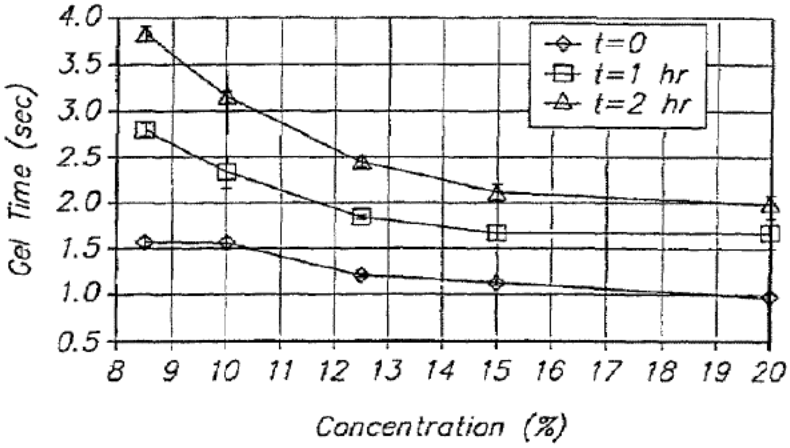
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Gel Time (sec)
t=0
t=1 hr
t=2 hr
8 9 10 11 12 13 14 15 16 17 18 19 20
Concentration (%)

FIG. 13

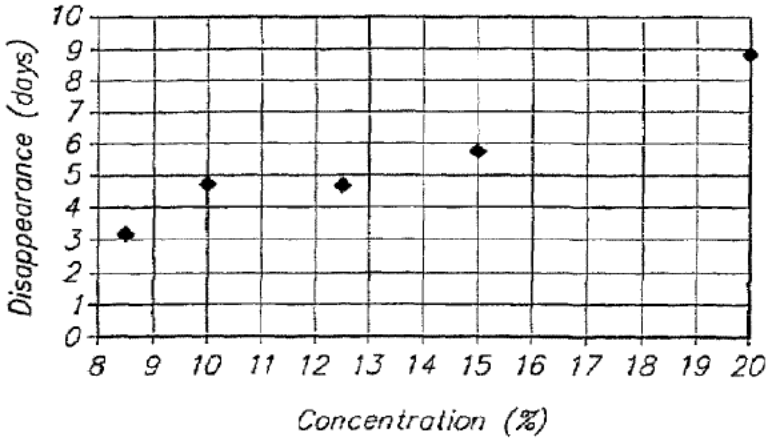
10
9
8
7
6
5
4
3
2
1
0
Disappearance (days)
8 9 10 11 12 13 14 15 16 17 18 19 20
Concentration (%)

FIG. 14

BIOCOMPATIBLE CROSSLINKED POLYMERS

The present patent application is a continuation in part of U.S. patent application Ser. No. 09/147,897, entitled "Methods And Devices For Preparing Protein Concentrates" filed Aug. 30, 1999 now abandoned which is a United States national stage application of Patent Cooperation Treaty application PCT/US/16897 filed Sep. 22, 1997 (publication number WO 98/12274), which has a priority date based on U.S. applications 60/026,526 filed Sep. 23, 1996; 60/039,904 filed Mar. 4, 1997; and 60/040,417 filed Mar. 13, 1997. The present patent application is also a continuation-in-part of U.S. patent application Ser. No. 09/454,900, filed Dec. 3, 1999 entitled "Biocompatible Crosslinked Polymers" now U.S. Pat. No. 6,566,406 which has a priority date based on U.S. patent application 60/110,849 filed Dec. 4, 1998. The present patent application claims priority to these other patents and patent applications which are hereby incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates generally to biocompatible crosslinked polymers, methods for preparing and using same.

BACKGROUND OF THE INVENTION

Almost every surgical treatment carries a risk that bodily tissues exposed during the course of the surgery will adhere to each other, a condition termed an adhesion. Gynecological and abdominal surgeries, in particular, are prone to causing adhesions, which often have the appearance of scar-like masses. Adhesions are frequently painful and are a significant cause of infertility resulting from gynecological surgeries. Adhesions caused by surgeries are often called surgical adhesions.

One approach to the treatment of adhesions has been to coat surgically exposed tissues with a gel before closing the surgical site. Gels of various types have been used, including suspensions of colloidal particles, and pastes of natural polymers. Various examples of some of these approaches are described in U.S. Pat. Nos. 6,020,326 and 5,605,938. Some of these approaches allow for the polymers to be added to the patient "in situ" in a solution and then chemically reacted inside the patient so that the polymers form covalent crosslinks to create a polymer network. This approach lets the polymer be formed in a way that closely conforms to the shape of the tissues in the body, as described, for example, in U.S. Pat. Nos. 5,410,016; 5,573,934 and 5,626,863.

Hydrogels are especially useful for use in the body because they are more biocompatible than non-hydrogels and are thus better tolerated in the body. Besides being useful for post-operative adhesions they can be used for many medical purposes, such as tissue augmentation, medical device coating, surgical sealing, and drug delivery. Examples of hydrogels formulated for such purposes are found in U.S. Pat. Nos. 4,414,976; 4,427,651; 4,925,677; 5,527,856; 5,550,188; and 5,814,621.

Crosslinked polymers have previously been formed using polymers equipped with either electrophilic or nucleophilic functional groups. For example, U.S. Pat. Nos. 5,296,518 and 5,104,909 to Grasel et al. describe the formation of crosslinked polymers from ethylene oxide rich prepolymers, wherein a polyisocyanate or low molecular weight diisocyanate is used as the electrophilic polymer or crosslinker, and

a polyoxyethylene based polyol with in situ generated amine groups is used as the nucleophilic precursor; see also U.S. Pat. Nos. 5,514,379; 5,527,856; and 5,550,188.

Polymeric hydrogels, for example, fibrin glue, crosslinked proteins, and crosslinked polyethylene oxides, are essentially colorless. This problem is often even more acute when the hydrogel is applied as a coating on a tissue because tissue coatings conventionally are thin. The resulting colorless solution or film is therefore difficult to visualize, especially in the typically wet and moist surgical environment. Under laparoscopic conditions, visibility is even more difficult due to the fact that only a two-dimensional view of the surgical field is available on the monitor that is used in such procedures.

SUMMARY OF THE INVENTION

The present inventors have realized that use of color in biocompatible crosslinked polymers and precursors greatly improves their performance in a surgical environment, especially under minimally invasive surgical procedures (MIS), e.g., laparoscopic, endoscopic. Moreover, the better visibility available with the use of color also permits efficient use of materials and avoids wastage.

An embodiment of the invention is a hydrogel for use on a substrate such as a patient's tissue. The hydrogel has water, a biocompatible visualization agent, and reactive hydrophilic polymers that form a crosslinked hydrogel after contact with the tissue. The hydrogel coats the tissue and forms a coating. The coating may have a free surface. The visualization agent is disposed in the hydrogel and reflects or emits light at a wavelength detectable to a human eye. This feature lets a user applying the hydrogel observe the hydrogel and estimate its thickness and apply the hydrogel until it reaches a predetermined thickness.

The hydrophilic polymers may be natural polymers, for example proteins e.g., collagen, fibrinogen, albumin, and fibrin, polysaccharides, or glycosaminoglycans,. The polymers can also have a hydrolytically biodegradable portion and/or a proteolytically degradable portion. The polymers are preferably covalently crosslinked and are crosslinkable via an electrophilic functional group-nucleophilic functional group reaction. An embodiment of the invention is a hydrogel that is coated onto a tissue and has a maximum thickness of between 0.1 to 10.0 mm.

Preferred biocompatible visualization agents are FD&C Blue #1, #2, #3, D&C Green #6, and methylene blue. The visualization agent may also be a fluorescent molecule. The visualization agent is preferably not covalently linked to the hydrogel.

Methods for using the polymeric compositions to coat a tissue include mixing hydrophilic precursor polymers with chemically distinct reactive functional groups such that they form crosslinks via nucleophilic-electrophilic reaction after mixing and contact with the tissue. The polymers crosslink to form a biodegradable hydrogel. A preferred application is to prevent surgical adhesions by applying the hydrogel as a coating on a tissue substrate and maintaining another surface of the hydrogel as a free surface. A visualization agent is preferably included so that the visualization agent is disposed within the hydrogel and reflects or emits light at a wavelength detectable to a human eye. A preferred method of use is to form a hydrogel on the tissue until the color and/or color intensity of the hydrogel indicates that a predetermined thickness of hydrogel has been deposited on the tissue.

An embodiment of the invention is a polymeric product made by a process of mixing hydrophilic polymers having nucleophilic functional groups with hydrophilic polymers having electrophilic functional groups such that they form a mix that crosslinks after contact with the tissue of a patient to form a biodegradable hydrogel that coats a tissue. In many applications it is desirable to also have a free surface. The hydrogel preferably contains a visualization agent in the mix of reactive precursor species so that the visualization agent is disposed within the interior and reflects or emits light at a wavelength detectable to a human eye.

An embodiment of the invention is a kit having a biocompatible visualization agent, at least two chemically distinct reactive precursor species, and instructions for using the visualization agent and the reactive precursor species such that the reactive precursor species may be combined to form crosslinked hydrophilic polymers that form a biodegradable hydrogel. In another embodiment, the visualization agent is premixed with one of the reactive precursor species.

It is an object of the present invention to provide biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers are formed without using free radical chemistry, and are formed using at least one non-toxic small molecule precursor.

It is another object of this invention to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers are formed from aqueous solutions, preferably under physiological conditions.

It is still another object of this invention to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers are formed in vivo.

It is a still further object of this invention to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers are biodegradable.

Another object of this invention is to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers, their precursors, or both are colored.

Another object of this invention is to provide methods for preparing tissue conforming, biocompatible crosslinked polymers in a desirable form, size and shape.

Another object of this invention is to provide methods for using biocompatible crosslinked polymers to form medically useful devices or implants for use as surgical adhesion prevention barriers, as implantable wound dressings, as scaffolds for cellular growth for tissue engineering or as surgical tissue adhesives or sealants.

Another object of this invention is to provide methods for using biocompatible crosslinked polymers to form medically useful devices or implants that can release bioactive compounds in a controlled manner for local, systemic, or targeted drug delivery.

Another object of this invention is to provide methods and compositions for producing composite biomaterials comprising fibers or particulates made of biodegradable biocompatible crosslinked polymers.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A–E depict electrophilic functional group water soluble and biodegradable crosslinkers or functional polymers, which can be crosslinked with appropriate nucleophilic functional group precursors.

FIG. 2F–J depict nucleophilic water soluble and biodegradable crosslinkers or functional polymers, which can be crosslinked with appropriate electrophilic precursors.

FIG. 3K–O depict electrophilic water soluble and biodegradable crosslinkers or functional polymers, which can be crosslinked with appropriate nucleophilic functional group precursors, wherein either the biodegradable linkages or the functional groups are selected so as to make the precursor water soluble.

FIG. 4P–T depict nucleophilic functional group water soluble crosslinkers or functional polymers, which can be crosslinked with appropriate electrophilic functional group precursors, and which are not biodegradable.

FIG. 5U–Y depict electrophilic water soluble crosslinkers or functional polymers, which can be crosslinked with appropriate nucleophilic functional group precursors, and which are not biodegradable.

FIG. 6 depicts the preparation of an electrophilic water soluble crosslinker or functional polymer using carbodiimide ("CDI") activation chemistry, its crosslinking reaction with a nucleophilic water soluble functional polymer to form a biocompatible crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 7 depicts the use of sulfonyl chloride activation chemistry to prepare an electrophilic functional polymer.

FIG. 8 depicts the preparation of an electrophilic water soluble crosslinker or functional polymer using N-hydroxysuccinimide ("NHS") activation chemistry, its crosslinking reaction with a nucleophilic water soluble functional polymer to form a biocompatible crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 9 depicts preferred NHS esters for use in the invention.

FIG. 10 shows the N-hydroxysulfosuccinimide ("SNHS") activation of a tetrafunctional sugar-based water soluble synthetic crosslinker and its crosslinking reaction with 4-arm amine terminated polyethylene glycol to form a biocompatible crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 11 shows the variation in gelation time with the number of amino groups for the reaction of 4 arm 10 kDa succinimidyl glutarate PEG ("SG-PEG") with di-, tri- or tetra-lysine.

FIG. 12 shows the variation in gelation time with the solution age of the electrophilic functional polymer.

FIG. 13 shows the variation in gelation time with the concentration of biocompatible crosslinked polymer precursors, and with the solution age of the 4 arm 10 kDa carboxymethyl-hydroxybutyrate-N-hydroxysuccinimidyl PEG ("CM-HBA-NS") electrophilic functional polymer.

FIG. 14 shows the variation in degradation time with the concentration of biocompatible crosslinked polymer.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have realized that use of color in biocompatible crosslinked polymers and/or reactive precursor species improves the performance of crosslinked networks of polymers and/or reactive precursor species in a surgical environment, especially for minimally invasive surgical (MIS) procedures. Many applications have the best results when an appropriate or predetermined amount of hydrogel is delivered to the surgical environment, for

example when applied to the surface of a substrate such as a tissue. A hydrogel that is too thick may reduce efficiency or interfere with other surgical aspects. For example, if a hydrogel is applied too thickly, it could interfere with closure of the wound or interfere with tissue movement, e.g., in intestinal applications. A hydrogel that is too thin will not serve its purpose, e.g., providing a barrier that prevents surgical adhesions or provides a strong seal against fluid leakage. The introduction of a visualization agent allows the user to determine the thickness of the applied hydrogel. The visualization agent is preferably an agent that provides a color that is visible to the human eye, e.g., a color that is detected visually by the user or detected by a video camera and relayed to a video screen observed by the user.

Conventional polymeric hydrogels may sometimes have a faint inherent color or develop a faint color as a result of chemical activity, but their lack of color makes a layer of a hydrogel very difficult to see after it has been applied to a tissue. Hydrogels have sometimes been mixed with image contrast agents to increase their visibility for medical imaging devices such as X-ray or magnetic resonance imaging (MRI) machines, as in, for example, U.S. Pat. No. 5,514,379. Colorants have also been used for hydrogels injected into bodily tissues, for example in U.S. Pat. Nos. 5,514,379 and 6,124,273.

The use of a visualization agent is especially preferred when a hydrogel is used to coat a substrate. A substrate coating surface is a surface of a hydrogel that contacts a substrate and, in the region of contact, is essentially in continuous contact with that substrate. Although some small portions of the coating or substrate may not be in contact, the contact is intimate. A substrate coating surface can be formed when the hydrogel crosslinks after contacting the substrate surface because the contact before crosslinking allows the hydrogel precursors to mix and conform to the shape of the substrate. A preformed hydrogel material generally does not have a substrate coating surface. A preferred substrate is a tissue of a patient.

A hydrogel with a substrate coating surface preferably also has a free surface when the hydrogel is used for prevention of adhesions. The hydrogel is applied to a tissue and crosslinks while having one free surface that is not adherent to any tissue but is instead freely movable relative to any tissues that it may subsequently contact. The free surface prevents the coated tissue from contact with other tissues and does not prevent the movement of other tissues so that protection and free movement are optimal. In this situation, a user that applies the hydrogel may observe the hydrogel by looking through the free surface into the hydrogel and at the coated tissue. A visualization agent in the hydrogel makes the hydrogel change in its appearance until the user determines that the thickness of the hydrogel is sufficient. For example, a blue dye in the hydrogel makes the hydrogel increasingly opaque as the thickness of the hydrogel increases.

It is preferable to provide color by adding a colored visualization agent to the hydrogel precursors before crosslinking. The coloring agent is thus present in a premixed amount that is already selected for the application. A preferred embodiment of the invention uses biocompatible crosslinked polymers formed from the reaction of precursors having electrophilic functional group and nucleophilic functional groups. The precursors are preferably water soluble, non-toxic and biologically acceptable.

Preferably, at least one of the precursors is a small molecule of about 1000 Da or less, and is referred to as a "crosslinker". The crosslinker preferably has a solubility of at least 1 g/100 mL in an aqueous solution. A crosslinked molecule may be crosslinked via an ionic or covalent bond, a physical force, or other attraction. Preferably, at least one of the other precursors is a macromolecule, and is referred to as a "functional polymer". The macromolecule, when reacted in combination with a crosslinker, is preferably at least five to fifty times greater in molecular weight than the small molecule crosslinker and is preferably less than about 60,000 Da. A more preferred range is a macromolecule that is seven to thirty times greater in molecular weight than the crosslinker and a most preferred range is about ten to twenty times difference in weight. Further, a macromolecular molecular weight of 5,000 to 50,000 is preferred, a molecular weight of 7,000 to 40,000 is more preferred and a molecular weight of 10,000 to 20,000 is most preferred. The term polymer, as used herein, means a molecule formed of at least three repeating groups. The term "reactive precursor species" means a polymer, functional polymer, macromolecule, small molecule, or crosslinker that can take part in a reaction to form a network of crosslinked molecules, e.g., a hydrogel.

An embodiment of the invention is a hydrogel for use on a patient's tissue that has water, a biocompatible visualization agent, and crosslinked hydrophilic polymers that form a hydrogel after contact with the tissue. The hydrogel coats the tissue and also has a free surface. The visualization agent reflects or emits light at a wavelength detectable to a human eye so that a user applying the hydrogel can observe the gel and also estimate its thickness.

Natural polymers, for example proteins or glycosaminoglycans, e.g., collagen, fibrinogen, albumin, and fibrin, may be crosslinked using reactive precursor species with electrophilic functional groups. Natural polymers are proteolytically degraded by proteases present in the body. Synthetic polymers and reactive precursor species are preferred, however, and may have electrophilic functional groups that are carbodiimidazole, sulfonyl chloride, chlorocarbonates, n-hydroxysuccinimidyl ester, succinimidyl ester or sulfasuccinimidyl esters. The term synthetic means a molecule that is not found in nature, e.g., polyethylene glycol. The nucleophilic functional groups may be, for example, amine, hydroxyl, carboxyl, and thiol. The polymers preferably have a polyalkylene glycol portion. More preferably they are polyethylene glycol based. The polymers preferably also have a hydrolytically biodegradable portion or linkage, for example an ester, carbonate, or an amide linkage. Several such linkages are well known in the art and originate from alpha-hydroxy acids, their cyclic dimmers, or other chemical species used to synthesize biodegradable articles, such as, glycolide, dl-lactide, l-lactide, caprolactone, dioxanone, trimethylene carbonate or a copolymer thereof. A preferred embodiment has reactive precursor species with two to ten nucleophilic functional groups each and reactive precursor species with two to ten electrophilic functional groups each. The hydrophilic species are preferably synthetic molecules.

Preferred biocompatible visualization agents are FD&C BLUE #1, FD&C BLUE #2, and methylene blue. These agents are preferably present in the final electrophilic-nucleophilic reactive precursor species mix at a concentration of more than 0.05 mg/ml and preferably in a concentration range of at least 0.1 to about 12 mg/ml, and more preferably in the range of 0.1 to 4.0 mg/ml, although greater concentrations may potentially be used, up to the limit of solubility of the visualization agent. These concentration ranges were found to give a color to the hydrogel that was desirable without interfering with crosslinking times (as

measured by the time for the reactive precursor species to gel). The visualization agent may also be a fluorescent molecule. The visualization agent is preferably not covalently linked to the hydrogel.

An embodiment of the invention is a hydrogel that is coated onto a tissue and generally has at least a portion with a thickness of between 0.8 to 12.0 mm. One technique for measuring the thickness is to create a hydrogel on a test surface and use a micrometer to measure thicknesses at various points. Alternatively, a calibrated videomicroscopic image could be used. The preferred thickness depends on the medical application but a preferred thickness for prevention of surgical adhesions is about 0.5 to 10.0 mm, and more preferably about 0.8 to 5 mm and even more preferably about 1–3 mm.

A preferred method of use is to form a hydrogel on the tissue until the color of the hydrogel indicates that a predetermined thickness of hydrogel has been deposited on the tissue. The deposition of the precursors that result in formation of the hydrogel may be by spraying, dripping, or delivery via a catheter. The user may apply the hydrogel to a test surface with a color that resembles the surface that the user contemplates using and observe the color that results when the hydrogel reaches a desired thickness that the user has predetermined. In use, the user applies the hydrogel until the desired color is reached. A typical patient's tissue has a pinkish appearance and the microvasculature can be observed as thin lines. One embodiment is to introduce a concentration of visualization agent into the hydrogel so that the user applies the hydrogel until the microvasculature is no longer visible through the hydrogel, at which point the hydrogel is a desired thickness. Another suitable method is to apply the hydrogel until the underlying tissue is obscured. An appropriately selected concentration of visualization agent is used so that the hydrogel obscures the tissue features when the hydrogel achieves a predetermined thickness. The predetermined thickness is chosen to correspond to the particular application. In these thickness evaluation approaches, a concentration that is too low will result in a hydrogel that is too thick and a concentration that is too high will result in a hydrogel that is too thin. Thus, the visualization agent allows the user to ascertain the presence of the hydrogel on the surface and also gain feedback on the appropriate thickness, preferably in combination with instructions provided as part of a kit. In some embodiments, suitable approaches can be used with visualization agents and polymers that crosslink by, for example, free radical polymerization, electrophilic functional group-nucleophilic functional group interaction.

An embodiment of the invention is a method of a user applying a hydrogel coating to a substrate and selecting a visually observable visualization agent to observe the hydrogel coating. The user may use visualization agents to see the hydrogel with the human eye or with the aid of an imaging device that detects visually observable visualization agents, e.g., a videocamera. A visually observable visualization agent is an agent that has a color detectable by a human eye. A characteristic of providing imaging to an X-ray or MRI machine is not a characteristic sufficient to establish function as a visually observable visualization agent. An alternative embodiment is a visualization agent that may not normally be seem by the human eye but is detectable at a different wavelength, e.g., the infra red or ultraviolet, when used in combination with a suitable imaging device, e.g., a videocamera.

A coating has a surface that can be viewed for use with a visually observable visualization agent. In contrast, a hydrogel injected into a blood vessel, muscle, or other tissue has essentially no surface for viewing a visualization agent because its surface area is essentially engaged with tissues of the patient. Further, polymers injected into a tissue lack a surface that is disposed on the surface of a tissue and do not provide a means for a user to control the thickness of the coating on the surface of the tissue. Hydrogels that are merely injected into a patient's body would not be equivalent to embodiments of the present invention that involve a hydrogel coating on a substrate and are inoperative for embodiments of the invention that entail use of a visualization agent in a hydrogel coating.

An embodiment of the invention involves a mixture or a process of mixing hydrophilic reactive precursor species having nucleophilic functional groups with hydrophilic reactive precursor species having electrophilic functional groups such that they form a mixture that crosslinks quickly after contact with the tissue of a patient to form a biodegradable hydrogel that coats and adheres to a tissue. This may be achieved by making reactive precursor species that crosslink quickly after mixing. Hydrophilic reactive precursor species can be dissolved in buffered water such that they provide low viscosity solutions that readily mix and flow when contacting the tissue. As they flow across the tissue, they conform to the shape of the small features of the tissue such as bumps, crevices and any deviation from molecular smoothness. If the reactive precursor species are too slow to crosslink, they will flow off the tissue and away into other portions of the body with the result that the user will be unable to localize the hydrogel on the desired tissue. Without limiting the invention to a particular theory of operation, it is believed that reactive precursor species s that crosslink appropriately quickly after contacting a tissue surface will form a three dimensional structure that is mechanically interlocked with the coated tissue. This interlocking contributes to adherence, intimate contact, and essentially continuous coverage of the coated region of the tissue.

Adherence is important for medical applications that require a coating, e.g., for prevention of adhesions, since a user must be able to place the hydrogel in the portions of the patient that are needful, for example, around an ovary or surrounding an intestine. Further, the hydrogel must remain on the intended tissue or it will be unable to provide a prophylactic barrier. The hydrogels of the invention have good adhesion onto tissue and are useful for all applications wherein surgical glues have previously been used. For example, sealing of the dura mater of the brain to prevent leakage of cerebrospinal fluid may be accomplished with combinations of reactive precursor species described herein by using reactive precursor species with nucleophilic functional groups for mixing with hydrophilic reactive precursor species having electrophilic functional groups to form a mix that crosslinks quickly after contact with the tissue of a patient, e.g., the dura mater, to form a hydrogel that coats a tissue.

A simple dip test that shows that a hydrogel has adherence. To perform this test, a gel of about 5×5 centimeters in lengthxwidth and about 4 to 10 mm in thickness is formed on a substrate, the hydrogel is immersed in water or physiological saline for five minutes, removed, and tilted to an angle of 90 degrees above horizontal, and dipped into and out of a vessel of physiological saline five times at a rate of about 10 mm per second so that the hydrogel passes through the air-water interface ten times. Then the substrate is rotated about 90 degrees so that the substrate is approximately horizontal and the hydrogel is below the substrate. The substrate is left in this position for five minutes. The gel

passes the dip test if less than about 1 square centimeter of the gel is then observed to be separated from the substrate. If the substrate lacks stiffness, it may be affixed to a stiff support so that it may tested. Physiological saline, in the context of the dip test, means a saline solution with an approximately physiological osmolarity and a pH of 7.0–7.4 at room temperature that is customarily used in cell culture, for example, phosphate buffered saline. As used herein, the gel has adherence to a substrate if it passes the dip test.

Suitable crosslinking times vary for different applications. In most applications, the crosslinking reaction leading to gelation occurs within about 10 minutes, more preferably within about 2 minutes, even more preferably within 10 seconds. In the case of most surgical adhesion prevention applications, it is preferable to use a hydrogel that crosslinks in less than about 10 seconds and more preferably in about 2–4 seconds in order to allow a user to make multiple passes with a hydrogel applicator tool such as a sprayer; see, for example commonly assigned U.S. Pat. Nos. 6,179,862; 6,165,201; 6,152,943; and U.S. patent applications Ser. No. 09/687,588, which are hereby incorporated herein by reference. In the case of tissues that can be accessed only indirectly, longer times are most preferable to allow the gel a longer time to flow into the inaccessible space. For example, application of an adhesion barrier in and around the spinal cord and exiting nerve roots following spine surgery may require several extra seconds to penetrate around the complex geometry of the tissues so that a preferred time is between about 5 and about 90 seconds and more preferably between about 10 and about 30 seconds. The Examples herein describe a variety of reactive precursor species and methods of making reactive precursor species that may be mixed to provide crosslinked networks that crosslink quickly after mixing such that one skilled in these arts will understand how to make the materials of the invention after reading this disclosure.

Functional Groups

Each precursor is multifunctional, meaning that it comprises two or more electrophilic or nucleophilic functional groups, such that a nucleophilic functional group on one precursor may react with an electrophilic functional group on another precursor to form a covalent bond. At least one of the precursors comprises more than two functional groups, so that, as a result of electrophilic-nucleophilic reactions, the precursors combine to form crosslinked polymeric products. Such reactions are referred to as "crosslinking reactions".

Preferably, each precursor comprises only nucleophilic or only electrophilic functional groups, so long as both nucleophilic and electrophilic precursors are used in the crosslinking reaction. Thus, for example, if a crosslinker has nucleophilic functional groups such as amines, the functional polymer may have electrophilic functional groups such as N-hydroxysuccinimides. On the other hand, if a crosslinker has electrophilic functional groups such as sulfosuccinimides, then the functional polymer may have nucleophilic functional groups such as amines or thiols. Thus, functional polymers such as proteins, poly(allyl amine), or amine-terminated di- or multifunctional poly(ethylene glycol) ("PEG") can be used.

Water Soluble Cores

The precursors preferably have biologically inert and water soluble cores. When the core is a polymeric region that is water soluble, preferred polymers that may be used include: polyether, for example, polyalkylene oxides such as polyethylene glycol("PEG"), polyethylene oxide ("PEO"), polyethylene oxide-co-polypropylene oxide ("PPO"), co-polyethylene oxide block or random copolymers, and polyvinyl alcohol ("PVA"); poly(vinyl pyrrolidinone) ("PVP"); poly(amino acids); dextran and proteins such as albumin. The polyethers and more particularly poly(oxyalkylenes) or poly(ethylene glycol) or polyethylene glycol are especially preferred. When the core is small molecular in nature, any of a variety of hydrophilic functionalities can be used to make the precursor water soluble. For example, functional groups like hydroxyl, amine, sulfonate and carboxylate, which are water soluble, maybe used to make the precursor water soluble. In addition, N-hydroxysuccinimide ("NHS") ester of subaric acid is insoluble in water, but by adding a sulfonate group to the succinimide ring, the NHS ester of subaric acid may be made water soluble, without affecting its reactivity towards amine groups.

Biodegradable Linkages

If it is desired that the biocompatible crosslinked polymer be biodegradable or absorbable, one or more precursors having biodegradable linkages present in between the functional groups may be used. The biodegradable linkage optionally also may serve as the water soluble core of one or more of the precursors. In the alternative, or in addition, the functional groups of the precursors may be chosen such that the product of the reaction between them results in a biodegradable linkage. For each approach, biodegradable linkages may be chosen such that the resulting biodegradable biocompatible crosslinked polymer will degrade or be absorbed in a desired period of time. Preferably, biodegradable linkages are selected that degrade under physiological conditions into non-toxic products.

The biodegradable linkage may be chemically or enzymatically hydrolyzable or absorbable. Illustrative chemically hydrolyzable biodegradable linkages include polymers, copolymers and oligomers of glycolide, dl-lactide, l-lactide, caprolactone, dioxanone, and trimethylene carbonate. Illustrative enzymatically5 hydrolyzable biodegradable linkages include peptidic linkages cleavable by metalloproteinases and collagenases. Additional illustrative biodegradable linkages include polymers and copolymers of poly (hydroxy acid)s, poly(orthocarbonate)s, poly(anhydride)s, poly(lactone)s, poly(aminoacid)s, poly(carbonate)s, and poly(phosphonate)s.

Visualization Agents

Where convenient, the biocompatible crosslinked polymer or precursor solutions (or both) may contain visualization agents to improve their visibility during surgical procedures. Visualization agents are especially useful when used in MIS procedures, due among other reasons to their improved visibility on a color monitor.

Visualization agents may be selected from among any of the various non-toxic colored substances suitable for use in medical implantable medical devices, such as FD&C BLUE dyes 3 and 6, eosin, methylene blue, indocyanine green, or colored dyes normally found in synthetic surgical sutures. The preferred color is green or blue because it has better visibility in presence of blood or on a pink or white tissue background. Red is the least preferred color, when used on a highly vascularized tissue that is red in color. However, red may be suitable when the underlying tissue is white, for example the cornea.

The visualization agent may be present with either reactive precursor species, e.g., a crosslinker or functional polymer solution. The preferred colored substance may or may not become chemically bound to the hydrogel.

The visualization agent may be used in small quantities, preferably less than 1% weight/volume, more preferably less that 0.01% weight/volume and most preferably less than 0.001% weight/volume concentration.

Additional visualization agents may be used, such as fluorescent (e.g., green or yellow fluorescent under visible light) compounds (e.g., fluorescein or eosin), x-ray contrast agents (e.g., iodinated compounds) for visibility under x-ray imaging equipment, ultrasonic contrast agents, or MRI contrast agents (e.g., Gadolinium containing compounds).

Visually observable visualization agents are preferred. Wavelengths of light from about 400 to 750 nm are observable to the human as colors (R. K. Hobbie, Intermediate Physics for Medicine and Biology, 2nd Ed., pages 371–373). Blue color is perceived when the eye receives light that is predominantly from about 450 to 500 nm in wavelength and green is perceived at about 500 to 570 nm (Id.). The color of an object is therefore determined by the predominant wavelength of light that it reflects or emits. Further, since the eye detects red or green or blue, a combination of these colors may be used to simulate any other color merely by causing the eye to receive the proportion of red, green, and blue that is perceived as the desired color by the human eye. Blue and green visualization agents are preferred since they are most readily visible when observing in situ crosslinking due to the approximately red color of the background color of tissue and blood. The color blue, as used herein, means the color that is perceived by a normal human eye stimulated by a wavelength of about 450 to 500 nm and the color green, as used herein, means the color that is perceived by a normal human eye stimulated by a wavelength of about 500 to 570 nm.

Crosslinking Reactions

The crosslinking reactions preferably occur in aqueous solution under physiological conditions. More preferably the crosslinking reactions occur "in situ", meaning they occur at local sites such as on organs or tissues in a living animal or human body. More preferably the crosslinking reactions do not release heat of polymerization. Preferably the crosslinking reaction leading to gelation occurs within about 10 minutes, more preferably within about 2 minutes, more preferably within about one minute, and most preferably within about 30 seconds. When it is desirable to build up a coating on a convex surface, the crosslinking reaction preferably occurs within about 2 minutes, more preferably in 30–60 seconds, and most preferably in 2–4 seconds.

Certain functional groups, such as alcohols or carboxylic acids, do not normally react with other functional groups, such as amines, under physiological conditions (e.g., pH 7.2–11.0, 37° C.). However, such functional groups can be made more reactive by using an activating group such as N-hydroxysuccinimide. Several methods for activating such functional groups are known in the art. Preferred activating groups include carbonyldiimidazole, sulfonyl chloride, aryl halides, sulfosuccinimidyl esters, N-hydroxysuccinimidyl ester, succinimidyl ester, epoxide, aldehyde, maleimides, imidoesters and the like. The N-hydroxysuccinimide esters or N-hydroxysulfosuccinimide groups are the most preferred groups for crosslinking of proteins or amine functionalized polymers such as amino terminated polyethylene glycol ("APEG").

FIGS. 1 to 5 illustrate various embodiments of preferred crosslinkers and functional polymers.

FIG. 1 illustrates possible configurations of degradable electrophilic crosslinkers or functional polymers. The biodegradable regions are represented by (^^^^^^) the functional groups are represented by (◄) and the inert water soluble cores are represented by (————). For crosslinkers, the central core is a water soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure A in FIG. 1 is a functional polymer, it is a linear water soluble and biodegradable functional polymer, end-capped with two functional groups (e.g., N-hydroxysuccinimide ester or NHS, epoxide or similar reactive groups). The water soluble core may be a polyalkylene oxide, preferably polyethylene glycol block copolymer, and it is extended with at least one biodegradable linkage between it and each terminal functional group. The biodegradable linkage may be a single linkage or copolymers or homopolymers of absorbable polymers such as polyhydroxy acids or polylactones.

When Structure B in FIG. 1 is a functional polymer it is a branched or star shaped biodegradable functional polymer which has an inert polymer at the center. Its inert and water soluble core is terminated with oligomeric biodegradable extensions, which in turn are terminated with reactive functional groups.

When Structures C and D in FIG. 1 are functional polymers, they are multifunctional 4 arm biodegradable functional polymers. This polymer again has a water-soluble soluble core at the center, which is a 4 arm, tetrafunctional polyethylene glycol (Structure C) or block copolyiner of PEO-PPO-PEO such as TETRONIC 908 (Structure D) which is extended with by small oligomeric extensions of biodegradable polymer to maintain water solubility and terminated with reactive functional end-groups such as CDI or NHS.

When Structure E in FIG. 1 is a functional polymer, it is a multifunctional star or graft type biodegradable polymer. This polymer has a water-soluble polymer like polyethylene oxide, polyvinyl alcohol or poly(vinyl pyrrolidinone) at the core which is completely or partially extended with biodegradable polymer. The biodegradable polymer is terminated with reactive end groups.

Structures A–E in FIG. 1 need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane etc. to form the resultant crosslinker. In addition, Structures A–E in FIG. 1 need not have polymeric biodegradable extensions, and the biodegradable extensions may consist of small molecules like succinate or glutarate or combinations of 2 or more esters, such as glycolate/2-hydroxybutyrate or glycolate/4-hydroxyproline, etc. A dimer or trimer of 4-hydroxyproline may be used not only to add degradability, but also to add nucleophilic functional group reactive sites via the pendant primary amines which are part of the hydroxyproline moiety.

Other variations of the core, the biodegradable linkage, and the terminal electrophilic group in Structures A–E in FIG. 1 may be constructed, so long as the resulting functional polymer has the properties of low tissue toxicity, water solubility, and reactivity with nucleophilic functional groups.

FIG. 2 illustrates various embodiments of nucleophilic biodegradable water soluble crosslinkers and functional polymers suitable for use with electrophilic functional polymers and crosslinkers described herein.

The biodegradable regions are represented by (^^^^^^); the functional groups are represented by (▮▮▮); and the inert water soluble cores are represented by (————). For crosslinkers, the central core is a water soluble small mol-

ecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure F in FIG. **2** is a functional polymer, it is a linear water soluble biodegradable polymer terminated with reactive functional groups like primary amine. The linear water-soluble core is a polyalkylene oxide, preferably polyethylene glycol block copolymer, which is extended with the biodegradable region which is a copolymer or homopolymers of polyhydroxy acids or polylactones. This biodegradable polymer is terminated with primary amines.

When Structure G in FIG. **2** is a functional polymer, it is a branched or star shaped biodegradable polymer which has an inert polymer at the center. The inert polymer is extended with single or oligomeric biodegradable extensions which are terminated with reactive functional groups.

When Structures H and I in FIG. **2** are functional polymers, they are multifunctional 4 arm biodegradable polymers. These polymers again have water-soluble cores at their center which are either a 4 arm, tetrafunctional polyethylene glycol (Structure II) or a block copolymer of PEO-PPO-PEO such as TETRONIC 908 (Structure I), extended with small oligomeric extensions of biodegradable polymers to maintain water solubility, and terminated with functional groups such as amines and thiols.

When Structure J in FIG. **2** is a functional polymer, it is a multifunctional star or graft type biodegradable polymer. This polymer has a water soluble polymer like polyethylene oxide, polyvinyl alcohol or poly(vinyl pyrrolidinone) at the core which is completely or partially extended with biodegradable polymer. The biodegradable polymer is terminated with reactive end groups.

Structures F–J in FIG. **2** need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane etc. to form the resultant crosslinker.

Other variations of the core, the biodegradable linkage, and the terminal nucleophilic functional group in Structures F–J in FIG. **2** may be constructed, so long as the resulting functional polymer has the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups.

FIG. **3** illustrates configurations of water-soluble electrophilic crosslinkers or functional polymers where the core is biodegradable. The biodegradable regions are represented by (^^^^^^) and the functional groups are represented by (◀). The biodegradable core is terminated with a reactive functional group that is also water solubilizing, such a N-hydroxysulfosuccinimide ester ("SNHS") or N-hydroxyethoxylated succinimide ester ("ENHS").

Structure K in FIG. **3** depicts a difunctional biodegradable polymer or oligomer terminated with SNHS or ENHS. The oligomers and polymers may be made of a poly(hydroxy acid) such as poly(lactic acid), which is insoluble in water. However, the terminal carboxylic acid group of these oligomers or polymers can be activated with N-hydroxysulfosuccinimide ester ("SNHS") or N-hydroxyethoxylated succinimide ester ("ENHS") groups. An ionic group, like a metal salt (preferably sodium salt) of sulfonic acid, or a nonionic group, like a polyethylene oxide on the succinimide ring, provides water-solubility while the NHS ester provides chemical reactivity towards amines. The sulfonate groups (sodium salts) or ethoxylated groups on the succinimide ring solubilize the oligomer or polymer without appreciably inhibiting reactivity towards amine groups.

Structures L–O in FIG. **3** represent multi-branched or graft type structures with terminal SNHS or ENHS group. The cores may comprise various non-toxic polyhydroxy compounds like sugars (xylitol, erythritol), glycerol, trimethylolpropane, which have been reacted with anhydrides such as succinic or glutaric anhydrides. The resultant acid groups were then activated with SNHS or ENHS groups to form water soluble crosslinkers or functional polymers.

FIG. **4** illustrates various nucleophilic functional polymers or crosslinkers that are not biodegradable. The nucleophilic functional groups are represented by (▮▮▮) and the inert water-soluble cores are represented by (————). For crosslinkers, the central core is a water-soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure P in FIG. **4** is a functional polymer it may be a water-soluble linear polymer such as polyethylene glycol terminated with reactive end group such as primary amines and thiols. Such polymers are commercially available from Sigma (Milwaukee, Wis.) and Shearwater Polymers (Huntsville, Ala). Some other preferred difunctional polymers ate PPO-PEO-PPO block copolymers such as PLURONIC F68 terminated with amine groups. PLURONIC or TETRONIC polymers are normally available with terminal hydroxyl groups. The hydroxyl groups are converted into amine groups by methods known in the art.

When Structures Q–T in FIG. **4** are functional polymers they may be multifunctional graft or branch type water soluble copolymers with terminal amine groups.

Structures P–T in FIG. **4** need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane, dilysine etc. to form the resultant crosslinker.

Other variations of the core and the terminal nucleophilic functional group in Structure P–T in FIG. **4** may be employed, so long as the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups are maintained.

FIG. **5** illustrates various electrophilic functional polymers or crosslinkers that are not biodegradable. The electrophilic functional groups are represented by (◀) and the inert water soluble cores are represented by (————). For crosslinkers, the central core is a water soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure U is a functional polymer, it may be a water-soluble polymer such as polyethylene glycol terminated reactive end group such as NHS or epoxide. Such polymers are commercially available from Sigma and Shearwater polymers. Some other preferred polymers are PPO-PEO-PPO block copolyiners such as PLURONIC F68 terminated with NTIS or SNHS group. PLURONIC or TETRONIC polymers are normally available with tenninal hydroxyl groups. The hydroxyl groups are converted into acid group by reacting with succinic anhydride. The terminated acid groups are reacted with N-hydroxysuccinimide in presence of DCC to generate NHS activated PLURONIC polymer.

When Structures V–Y are functional polymers they may be multifunctional graft or branch type PEO or PEO block copolymers (TETRONICS) activated with terminal reactive groups such as NHS.

Structures U–Y in FIG. **5** need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, tetraglycerol, hexaglycerol, inositol, trimethylolpropane, dilysine etc. to form the resultant crosslinker.

Other variations of the core and the terminal nucleophilic functional group in Structures U–Y in FIG. **5** may be employed, so long as the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups are maintained.

Preparation of Structures A–Y in FIGS. **1–5**

The polymeric crosslinkers and functional polymers illustrated as Structures A–Y in FIGS. **1** to **5** may be prepared using variety of synthetic methods. Their preferred compositions are described in Table 1.

TABLE 1

Preferred Crosslinkers and Functional Polymers

Structure	Brief Description	Typical Example
A	Water soluble, linear difunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences which are cleavable by enzymes and terminated with protein reactive functional groups	Polyethylene glycol or ethoxylated propylene glycol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
B	Water soluble, trifuncational crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Ethoxylated glycerol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
C	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	4 arm polyethylene glycol, erythritol or pentaerythritol or pentaerythritol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
D	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like TETRONIC 908 chain extended with oligotrimethylene carbonate and terminated with N-hydroxysuccinimide ester
E	Water soluble, branched crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Low molecular weight polyvinyl alcohol with 1% to 20% hydroxyl groups extended with oligolactate and terminated with N-hydroxysuccinimide ester
F	Water soluble, liner difunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer surfactant like PLURONIC F68 chain extended with oligolactate and terminated with amino acids such as lysine or peptide sequences that may contain two amine groups
G	Water soluble, trifunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Ethoxylated glycerol chain extended with oligolactate and terminated with aminoacid such as lysine
H	Water soluble, tetrafuncational crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide	4 arm polyethylene glycol or tetra erythritol chain extended with oligolactate and terminated with aminoacid such as lysine

TABLE 1-continued

Preferred Crosslinkers and Functional Polymers

Structure	Brief Description	Typical Example
	sequences and terminated with amines, carboxylic acid or thiols	
I	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like TETRONIC 908 chain extended with oligotrimethylene carbonate and terminated with aminoacid such as lysine.
J	Water soluble, multifunctional or graft type crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Low molecular weight polyvinyl alcohol with 1–20% hydroxyl groups extended with oligolactate and terminated with aminoacid such as lysine
K	Water soluble, linear difunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Difunctional oligolactic acid with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
L	Water soluble branched trifunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Trifunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
M	Water soluble, branched tetrafunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Tetrafunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
N	Water soluble, branched tetrafunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Tetrafunctional oligocaprolatone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydruxysuccinimide ester.
O	Water soluble, branched multifunctional crosslinker or functional polymer such as oligomers f hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Multifunctional oligolactic acid with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydroxysuccinimide ester.
P	Water soluble, linear difunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	Polyethylene glycol with terminal amines groups
Q	Water soluble, branched trifunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols as functional group	Ethoxylated glycerol with terminal amines groups
R	Water soluble, branched tetrafunctional crosslinker of functional polymer terminated with amines, carboxylic acid or thiols functional groups	4 arm polyethylene glycol modified to produce terminal amine groups
S	Water soluble, branched tetrafunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polyprophylene oxide-polyethylene oxide block copolymer like Tetronic 908 modified to generate terminal amine groups

TABLE 1-continued

Preferred Crosslinkers and Functional Polymers

Structure	Brief Description	Typical Example
T	Water soluble, branched or graft crosslinker or functional polymer with terminal amines, carboxylic acid or thiols functional groups	Polylysine, albumin, polyallyl amine
U	Water soluble, linear difunctional crosslinker or functional polymer terminated with protein reactive functional groups	Polylysine, albumin, polyallyl amine
V	Water soluble branched trifunctional crosslinker or functional polymer terminated with protein reactive functional groups	Ethoxylated glycerol terminated with n-hydroxysuccinimide
W	Water soluble branched tetrafunctional crosslinker or functional polymer terminated with protein reactive functional groups	4 arm polyethylene glycol terminated with n-hydroxysuccinimide esters
X	Water soluble branched tetrafunctional crosslinker or functional polymer terminated with protein reactive functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 with n-hydroxysuccinimide ester as end group
Y	Water soluble, branched or graft polymer crosslinker functional polymer with protein reactive functional groups	Poly (vinyl pyrrolidinone)-co-poly or (n-hydroxysuccinimide acrylate) copolymer (9:1), molecular weight < 40000 Da

First, the biodegradable links of Structures A–J in FIGS. **1** and **2** may be composed of specific di or multifunctional synthetic amino acid sequences which are recognized and cleaved by enzymes such as collagenase, and may be synthesized using methods known to those skilled in the peptide synthesis art. For example, Structures A–E in FIG. **1** may be obtained by first using carboxyl, amine or hydroxy terminated polyethylene glycol as a starting material for building a suitable peptide sequence. The terminal end of the peptide sequence is converted into a carboxylic acid by reacting succinic anhydride with an appropriate amino acid. The acid group generated is converted to an NHS ester by reaction with N-hydroxysuccinimide.

The functional polymers described in FIG. **2** may be prepared using a variety of synthetic methods. In a preferred embodiment, the polymer shown as Structure F may be obtained by ring opening polymerization of cyclic lactones or carbonates initiated by a dihydroxy compound such as PLURONIC F 68 in the presence of a suitable catalyst such as stannous 2-ethyihexanoate. The molar equivalent ratio of caprolactone to PLURONIC is kept below 10 to obtain a low molecular weight chain extension product so as to maintain water solubility. The terminal hydroxyl groups of the resultant copolymer are converted into amine or thiol by methods known in the art.

In a preferred method, the hydroxyl groups of a PLURONIC-caprolactone copolymer are activated using tresyl chloride. The activated groups are then reacted with lysine to produce lysine tenminated PLURONIC-caprolactone copolymer. Alternatively, an amine-blocked lysine derivative is reacted with the hydroxyl groups of a PLURONIC-caprolactone copolymer and then the amine groups are regenerated using a suitable deblocking reaction.

Structures G, H, I and J in FIG. **2** may represent multifunctional branched or graft type copolymers having water soluble core extended with oligohydroxy acid polymer and terminated with amine or thiol groups.

For example, in a preferred embodiment, the functional polymer illustrated as Structure G in FIG. **2** is obtained by ring opening polymerization of cyclic lactones or carbonates initiated by a tetrahydroxy compound such as 4 arm, tetrahydroxy polyethylene glycol (molecular weight 10,000 Da), in the presence of a suitable catalyst such as stannous octoate. The molar equivalent ratio of cyclic lactone or carbonate to PEG is kept below 10 to obtain a low molecular weight extension, and to maintain water solubility (polymers of cyclic lactones generally are not as water soluble as PEG). Alternatively, hydroxyacid as a biodegradable link may be attached to the PEG chain using blocking/deblocking chemistry known in the peptide synthesis art. The terminal hydroxy groups of the resultant copolymer are activated using a variety of reactive groups known in the art. The CDI activation chemistry and sulfonyl chloride activation chemistry is shown in FIGS. **6** and **7**, respectively.

The most preferred reactive groups are N-hydroxysuccinimide esters, synthesized by any of several methods. In a preferred method, hydroxyl groups are converted to carboxylic groups by reacting them with anhydrides such as succinic anhydride in the presence of tertiary amines such as pyridine or triethylamine or dimethylaminopyridine ("DMAP"). Other anhydrides such as glutaric anhydride, phthalic anhydride, maleic anhydride and the like may also be used. The resultant terminal carboxyl groups are reacted with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide ("DCC") to produce N-hydroxysuccinimide ester (referred as NHS activation). The NHS activation and

crosslinking reaction scheme is shown in FIG. **8**. The most preferred N-hydroxysuccinimide esters are shown in FIG. **9**.

In a preferred embodiment, the polymer shown as structure H is obtained by ring opening polymerization of glycolide or trimethylene carbonate initiated by a tetrahydroxy compound such as tetrafunctional polyethylene glycol (molecular weight 2000 Da) in the presence of a catalyst such as stannous 2-ethylhexoate. The molar equivalent ratio of glycolide to PEG is kept from 2 to 10 to obtain a low molecular weight extension. The terminal hydroxy groups of the resultant copolymer are converted into amine groups by reaction with lysine as mentioned previously. Similar embodiments can be obtained using analogous chain extension synthetic strategies to obtain structures F, G, I and J by starting with the appropriate corresponding polyol.

Structures K, L, M, N and O in FIG. **3** are made using a variety of synthetic methods. In a preferred embodiment, the polymer shown as Structure L in FIG. **3** is obtained by ring opening polymerization of cyclic lactones by a trihydroxy compound such as glycerol in the presence of a catalyst such as stannous 2-ethylhexanoate. The molar equivalent ratio of cyclic lactone to glycerol is kept below 2, so that only low molecular weight oligomers are obtained. The low molecular weight oligomer ester is insoluble in water. The terminal hydroxy groups of the resultant copolymer are activated using N-hydroxysulfosuccinimide groups. This is achieved by converting hydroxy groups to carboxylic groups by reacting with anhydrides such as succinic anhydride in presence of tertiary amines. The resultant terminal carboxyl groups are reacted with N-hydroxysulfosuccinimide or N-hydroxyethoxylated succinimide in the presence of dicyclohexylcarbodiimide ("DCC") to produce a sulfonated or ethoxylated NHS ester. The sulfonate or PEO chain on the succinimide ring gives water solubility to the oligoester.

The foregoing method generally is applied to solubilize only low molecular weight multi-branched oligoesters, with molecular weights below 1000. In another variation of this method, various non-toxic polyhydroxy compounds, preferably sugars, such as erythritol, xylitol are reacted with succinic anhydride in the presence of a tertiary amine. The terminal carboxyl group of succinated erythritol is esterified with N-hydroxysulfosuccinimide (FIG. **9**). Similar embodiments may be obtained using analogous synthetic strategies to obtain structures K, and M–O by starting with the appropriate starting materials.

Structures P–R may be synthesized by reacting the appropriate starting material, such as a linear (P) or 2- or 3-arm branched PEG (Q, R) with hydroxy end groups, with lysine as mentioned previously, such that the arms of the PEG oligomers are capped with amine end groups. Structure S may be synthesized, using a multistep reaction, from PEG, glycerol and a diisocyanate. In the first step a PEG diol is reacted with excess diisocyanate, such as 4,4'diphenyl methane diisocyanate ("MDI"), methylene-bis (4-cyclohexylisocyanate) ("HMDI") or hexamethylenediisocyanate ("HDI"). After purification the resultant PEG diisocyanate is added dropwise to excess glycerol or trimethylol propane or other triol and reacted to completion. The purified product, now having diol end groups, is again reacted with excess diisocyanate and purified, yielding a PEG-tetra-isocyanate. This tetrafunctional PEG subsequently may be reacted with excess PEG diols, yielding a 4 arm PEG synthesized from a PEG diol oligomer. In the final step lysine end groups are incorporated, as discussed previously.

Structure T may be synthesized as follows: First synthesize a random copolymer of PEG-monoacrylate and some other acrylate or combination of acrylates, such that the final polyacrylate is water soluble. Other acrylates include, but are not limited to, 2-hydroxyethylacrylate, acrylic acid, and acrylamide. Conditions may be varied to control the molecular weight as desired. In the final step, the acrylate is reacted with lysine as discussed previously, using an appropriate quantity to achieve the desired degree of amination.

One method of synthesizing Structures U–Y is to use dicyclohexylcarbodiimide coupling to a carboxylate end group. For Structures U–W, one can react the appropriate PEG-diol, -triol or -tetra-hydroxy starting material with excess succinic anhydride or glutaric anhydride such that all end groups are effectively carboxylated. Structures X and Y may be made in a manner similar to that used for Structures S and T, except that in the last step instead of end capping with lysine, end capping with succinic anhydride or glutaric anhydride is performed.

Preparation of Biocompatible Polymers

Several biocompatible crosslinked hydrogels may be produced using the crosslinkers and functional polymers described in FIGS. **1** to **5**. Preferred combinations of such polymers suitable for producing such biocompatible crosslinked polymers are described in Table 2. In Table 2, the crosslinker functional groups are N-hydroxy succinimide esters and the functional polymer functional groups are primary amines.

TABLE 2

Biocompatible Polymers Synthesized from Crosslinkers and Functional Polymers of Table 1

Crosslinker Structure	Functional Polymer Structure	Concentration	Medium
B or C	H and R	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7–10
A, B or C	H, P, Q, R and S	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7–10
Y	T, H, P and Q	Molar Equivalent; > 10% W/V	Borate or triethanol amine buffer, pH 7–910
W, V	H and J	Molar Equivalent; > 20% W/V	Bicarbonate buffer, pH 7–10

TABLE 2-continued

Biocompatible Polymers Synthesized from Crosslinkers and Functional Polymers of Table 1

Crosslinker Structure	Functional Polymer Structure	Concentration	Medium
X	I, J and H	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7–10

The reaction conditions for crosslinking will depend on the nature of the functional groups. Preferred reactions are conducted in buffered aqueous solutions at pH 5 to 12. The preferred buffers are sodium borate buffer (pH 10) and triethanol amine buffer (pH 7). Elevated pH increases the speed of electrophilic-nucleophilic reactions. In some embodiments, organic solvents such as ethanol or isopropanol may be added to improve the reaction speed or to adjust the viscosity of a given formulation.

The synthetic crosslinked gels described above degrade due to hydrolysis of the biodegradable region. The degradation of gels containing synthetic peptide sequences will depend on the specific enzyme and its concentration. In some cases, a specific enzyme may be added during the crosslinking reaction to accelerate the degradation process.

When the crosslinker and functional polymers are synthetic (for example, when they are based on polyalkylene oxide), then it is desirable and in some cases essential to use molar equivalent quantities of the reactants. In some cases, molar excess crosslinker may be added to compensate for side reactions such as reactions due to hydrolysis of the functional group.

When choosing the crosslinker and crosslinkable polymer, at least one of polymers must have more than 2 functional groups per molecule and at least one degradable region, if it is desired that the resultant biocompatible crosslinked polymer be biodegradable. For example, the difunctional crosslinker shown as Structure A in FIG. **1** cannot form a crosslinked network with the difunctional polymers shown as Structure F in FIG. **2** or Structure P in FIG. **4**. Generally, it is preferred that each biocompatible crosslinked polymer precursor have more than 2 and more preferably 4 or more functional groups.

Preferred electrophilic functional groups are NHS, SNHS and ENHS (FIG. **9**). Preferred nucleophilic functional groups are primary amines. The advantage of the NHS-amine reaction is that the reaction kinetics lead to quick gelation usually within 10 about minutes, more usually within about 1 minute and most usually within about 10 seconds. This fast gelation is preferred for in situ reactions on live tissue.

The NHS-amine crosslinking reaction leads to formation of N-hydroxysuccinimide as a side product. The sulfonated or ethoxylated forms of N-hydroxysuccinimide are preferred due to their increased solubility in water and hence their rapid clearance from the body. The sulfonic acid salt on the succinimide ring does not alter the reactivity of NHS group with the primary amines.

The NHS-amine crosslinking reaction may be carried out in aqueous solutions and in the presence of buffers. The preferred buffers are phosphate buffer (pH 5.0–7.5). triethanolamine buffer (pH 7.5–9.0) and borate buffer (pH 9.0–12) and sodium bicarbonate buffer (pH 9.0–10.0).

Aqueous solutions of NHS based crosslinkers and functional polymers preferably are made just before the crosslinking reaction due to reaction of NHS groups with water. Longer "pot life" may be obtained by keeping these solutions at lower pH (pH 4–5).

The crosslinking density of the resultant biocompatible crosslinked polymer is controlled by the overall molecular weight of the crosslinker and functional polymer and the number of functional groups available per molecule. A lower molecular weight between crosslinks such as 600 will give much higher crosslinking density as compared to a higher molecular weight such as 10,000. Higher molecular weight functional polymers are preferred, preferably more than 3000 so as to obtain elastic gels.

The crosslinking density also may be controlled by the overall percent solids of the crosslinker and functional polymer solutions. Increasing the percent solids increases the probability that an electrophilic functional group will combine with a nucleophilic functional group prior to inactivation by hydrolysis. Yet another method to control crosslink density is by adjusting the stoichiometry of nucleophilic functional groups to electrophilic functional groups. A one to one ratio leads to the highest crosslink density.

Preparation of Biodegradable Polymers

The biodegradable crosslinkers described in FIGS. **1** and **3** may be reacted with proteins, such as albumin, other serum proteins, or serum concentrates to generate crosslinked polymeric networks. Briefly, aqueous solutions of the crosslinkers described in FIG. **1** and FIG. **3** (at a concentration of 50 to 300 mg/ml) are mixed with concentrated solutions of albumin (600 mg/ml) to produce a crosslinked hydrogel. This reaction can be accelerated if a buffering agent, e.g., borate buffer or triethanol amine, is added during the crosslinking step.

The resultant crosslinked hydrogel is a semisynthetic hydrogel whose degradation depends on the degradable segment in the crosslinker as well as degradation of albumin by enzymes. In the absence of any degradable enzymes, the crosslinked polymer will degrade solely by the hydrolysis of the biodegradable segment. If polyglycolate is used as the biodegradable segment, the crosslinked polymer will degrade in 1–30 days depending on the crosslinking density of the network. Similarly, a polycaprolactone based crosslinked network will degrade in 1–8 months. The degradation time generally varies according to the type of degradable segment used, in the following order: polyglycolate<polylactate<polytrimethylene carbonate<polycaprolactone. Thus it is possible to construct a hydrogel with a desired degradation profile, from a few days to months, using a proper degradable segment.

The hydrophobicity generated by biodegradable blocks such as oligohydroxy acid blocks or the hydrophobicity of PPO blocks in PLURONIC or TETRONIC polymers are

helpful in dissolving small organic drug molecules. Other properties which will be affected by incorporation of biodegradable or hydrophobic blocks are: water absorption, mechanical properties and thermosensitivity.

Methods of Using Biocompatible Polymers

The biocompatible crosslinked polymers and their precursors described above may be used in a variety of applications, such as components of tissue adhesives, tissue sealants, drug delivery vehicles, wound covering agents, barriers in preventing postoperative adhesions, and others. These and other suitable applications are reviewed in Schlag and Redl, "Fibrin Sealant" in *Operative Surgery*, volumes 1–7 (1986), which is incorporated herein by reference.

In Situ Formation

In many applications, the biocompatible crosslinked polymers of this invention typically will be formed "in situ" at a surgical site in the body. The various methodologies and devices for performing "in situ" gelation, developed for other adhesive or sealant systems such fibrin glue or sealant applications, may be used with the biocompatible crosslinked polymers of this invention. Thus, in one embodiment, an aqueous solution of a freshly prepared crosslinker (e.g., SNHS-terminated oligolactide synthesized from a glycerol core in phosphate buffered saline ("PBS") at pH 5 to 7.2) and a functional polymer (e.g., albumin or amine terminated tetrafunctional polyethylene glycol at pH 10 in sodium borate) are applied and mixed on the tissue using a double barrel syringe (one syringe for each solution). The two solutions may be applied simultaneously or sequentially. In some embodiments, it is preferred to apply the precursor solutions sequentially so as to "prime" the tissue, resulting in improved adherence of the biocompatible crosslinked polymer to the tissue. Where the tissue is primed, the crosslinker precursor is preferably applied to the tissue first, followed by the functional polymer solution.

One may use specialized devices to apply the precursor solutions, such as those described in U.S. Pat. Nos. 4,874,368; 4,631,055; 4,735,616; 4,359,049; 4,978,336; 5,116,315; 4,902,281; 4,932,942; Published Patent Cooperation Treaty Patent Application No. WO 91/09641; and R. A. Tange, "Fibrin Sealant" in *Operative Medicine: Otolaryngology*, volume 1 (1986), the disclosures of which are herein incorporated by reference.

Drug Delivery

The subject crosslinkers, functional polymer and their reaction products, the crosslinked materials advantageously may be used for localized drug therapy. Biologically active agents or drug compounds that may be added and delivered from the crosslinked polymer or gel include: proteins, glycosaminoglycans, carbohydrates, nucleic acid, inorganic and organic biologically active compounds where specific biologically active agents include but are not limited to: enzymes, antibiotics, antineoplastic agents, local anesthetics, hormones, angiogenic agents, anti-angiogenic agents, growth factors, antibodies, neurotransmitters, psychoactive drugs, anticancer drugs, chemotherapeutic drugs, drugs affecting reproductive organs, genes, and oligonucleotides.

To prepare such crosslinked composition, the bioactive compounds described above are mixed with the crosslinkable polymer prior to making the aqueous solution or during the aseptic manufacturing of the functional polymer. This mixture then is mixed with the crosslinker to produce a crosslinked material in which the biologically active substance is entrapped. Functional polymers made from inert polymers like PLURONIC, TETRONICS or Tween" surfactants are preferred in releasing small molecule hydrophobic drugs.

In a preferred embodiment, the active agent or agents are present in a separate phase when crosslinker and crosslinkable polymers are reacted to produce a crosslinked polymer network or gel. This phase separation prevents participation of bioactive substance in the chemical crosslinking reaction such as reaction between NHS ester and amine group. The separate phase also helps to modulate the release kinetics of active agent from the crosslinked material or gel, where 'separate phase' could be oil (oil-in water emulsion), biodegradable vehicle, and the like. Biodegradable vehicles in which the active agent may be present include: encapsulation vehicles, such as microparticles, microspheres, microbeads, micropellets, and the like, where the active agent is encapsulated in a bioerodable or biodegradable polymers such as polymers and copolymers of: poly(anhydride), poly (hydroxy acid)s, poly(lactone)s, poly(trimethylene carbonate), poly(glycolic acid), poly(lactic acid), poly(glycolic acid)-co-poly(glycolic acid), poly(orthocarbonate), poly(caprolactone), crosslinked biodegradable hydrogel networks like fibrin glue or fibrin sealant, caging and entrapping molecules, like cyclodextrin, molecular sieves and the like. Microspheres made from polymers and copolymers of poly (lactone)s and poly(hydroxy acid) are particularly preferred as biodegradable encapsulation vehicles.

In using crosslinked materials which are described herein as drug delivery vehicles, the active agent or encapsulated active agent may be present in solution or suspended form in crosslinker component or functional polymer solution component. The nucleophilic component, whether it be in the crosslinker or the functional polymer is the preferred vehicle due to absence of reactive groups. The functional polymer along with bioactive agent, with or without encapsulating vehicle, is administered to the host along with equivalent amount of crosslinker and aqueous buffers. The chemical reaction between crosslinker and the functional polymer solution readily takes place to form a crosslinked gel and acts as a depot for release of the active agent to the host. Such methods of drug delivery find use in both systemic and local administration of an active agent.

In using the crosslinked composition for drug delivery as mentioned above, the amount of crosslinkable polymer, crosslinker and the dosage agent introduced in the host will necessarily depend upon the particular drug and the condition to be treated. Administration may be by any convenient means such as syringe, canula, trocar, catheter and the like.

Several methods for the formation of regional adhesion barriers are described, in which any of a variety of water soluble macromeric precursors are used. The term "macromeric precursor" or "macromer" is meant to connote an oligomeric or polymeric molecule that contains functional groups that enable further crosslinking. Preferably the functionality of a macromer molecule is >2 so that a crosslinked network or hydrogel results upon crosslinking.

In one embodiment, a crosslinked regional barrier is formed in situ, for example, by electrophilic-nucleophilic reaction, free radical polymerization initiated by a redox system or thermal initiation, wherein two components of an initiating system are simultaneously, sequentially or separately instilled in a body cavity to obtain widespread dispersal and coating of all or most visceral organs within that cavity prior to gelation and crosslinking of the regional barrier. Once the barrier is formed, the organs remain

isolated from each other for a predetermined period, depending upon the absorption profile of the adhesion barrier material.

Preferably, the barrier is selected to have a low stress at break in tension or torsion, so as to not adversely affect normal physiological function of visceral organs within the region of application. The barrier also may contain a drug or other therapeutic agent.

Certain embodiments of the invention are accomplished by providing compositions and methods to control the release of relatively low molecular weight therapeutic species using hydrogels. In accordance with the principles of the present invention, a therapeutic species first is dispersed or dissolved within one or more relatively hydrophobic rate modifying agents to form a mixture. The mixture may be formed into microparticles, which are then entrapped within a bioabsorbable hydrogel matrix so as to release the water soluble therapeutic agents in a controlled fashion. Alternatively, the microparticles may be formed in situ during crosslinking of the hydrogel.

In one method of the present invention, hydrogel microspheres are formed from polymerizable macromers or monomers by dispersion of a polymerizable phase in a second immiscible phase, wherein the polymerizable phase contains at least one component required to initiate polymerization that leads to crosslinking and the immiscible bulk phase contains another component required to initiate crosslinking, along with a phase transfer agent. Pre-formed microparticles containing the water soluble therapeutic agent may be dispersed in the polymerizable phase, or formed in situ, to form an emulsion. Polymerization and crosslinking of the emulsion and the immiscible phase is initiated in a controlled fashion after dispersal of the polymerizable phase into appropriately sized microspheres, thus entrapping the microparticles in the hydrogel microspheres. Visualization agents may be included, for instance, in the microspheres, microparticles, and/or microdroplets.

Embodiments of the invention include compositions and methods for forming composite hydrogel-based matrices and microspheres having entrapped therapeutic compounds. In one embodiment, a bioactive agent is entrapped in microparticles having a hydrophobic nature (herein called "hydrophobic microdomains"), to retard leakage of the entrapped agent. More preferably, the composite materials that have two phase dispersions, where both phases are absorbable, but are not miscible. For example, the continuous phase may be a hydrophilic network (such as a hydrogel, which may or may not be crosslinked) while the dispersed phase may be hydrophobic (such as an oil, fat, fatty acid, wax, fluorocarbon, or other synthetic or natural water immiscible phase, generically referred to herein as an "oil" or "hydrophobic" phase).

The oil phase entraps the drug and provides a barrier to release by slow partitioning of the drug into the hydrogel. The hydrogel phase in turn protects the oil from digestion by enzymes, such as lipases, and from dissolution by naturally occurring lipids and surfactants. The latter are expected to have only limited penetration into the hydrogel, for example, due to hydrophobicity, molecular weight, conformation, diffusion resistance, etc. In the case of a hydrophobic drug which has limited solubility in the hydrogel matrix, the particulate form of the drug may also serve as the release rate modifying agent.

Hydrophobic microdomains, by themselves, may be degraded or quickly cleared when administered in vivo, making it difficult to achieve prolonged release directly using microdroplets or microparticles containing the entrapped agent in vivo. In accordance with the present invention, however, the hydrophobic microdomains are sequestered in a gel matrix. The gel matrix protects the hydrophobic microdomains from rapid clearance, but does not impair the ability of the microdroplets or microparticles to release their contents slowly. Visualization agents may be included, for instance, in the gel matrix or the microdomains.

In one embodiment, a microemulsion of a hydrophobic phase and an aqueous solution of a water soluble molecular compound, such as a protein, peptide or other water soluble chemical is prepared. The emulsion is of the "water-in-oil" type (with oil as the continuous phase) as opposed to an "oil-in-water" system (where water is the continuous phase). Other aspects of drug delivery are found in commonly assigned U.S. patent applications Ser. No. 09/134,287 entitled "Composite Hydrogel Drug Delivery Systems"; Ser. No 09/390,046 entitled "Methods and Apparatus for Intraluminal Deposition of Hydrogels"; and Ser. No 09/134,748 entitled "Methods for Forming Regional Tissue Adherent Barriers and Drug Delivery Systems", each of which are hereby incorporated by reference.

In another aspect of the present invention, the hydrogel microspheres are formed having a size that will provide selective deposition of the microspheres, or may linked with ligands that target specific regions or otherwise affect deposition of the microspheres within a patient's body.

Controlled rates of drug delivery also may be obtained with the system of the present invention by degradable, covalent attachment of the bioactive molecules to the crosslinked hydrogel network. The nature of the covalent attachment can be controlled to enable control of the release rate from hours to weeks or longer. By using a composite made from linkages with a range of hydrolysis times, a controlled release profile may be extended for longer durations.

Composite Biomaterials

The biocompatible crosslinked polymers of this invention optionally may be reinforced with flexible or rigid fibers, fiber mesh, fiber cloth and the like. The insertion of fibers improves mechanical properties like flexibility, strength, and tear resistance. In implantable medical applications, biodegradable fibers, cloth, or sheets made from oxidized cellulose or poly(hydroxy acid)s polymers like polylactic acid or polyglycolic acid, are preferred. Such reinforced structures may be produced using any convenient protocol known in the art.

In a preferred method, aqueous solutions of functional polymers and crosslinkers are mixed in appropriate buffers and proportions are added to a fiber cloth or net such as INTERCEED (Ethicon Inc., New Brunswick, N.J.). The liquid mixture flows into the interstices of the cloth and becomes crosslinked to produce a composite hydrogel. Care is taken to ensure that the fibers or fiber mesh are buried completely inside the crosslinked hydrogel material. The composite structure can be washed to remove side products such as N-hydroxysuccinimide. The fibers used are preferably hydrophilic in nature to ensure complete wetting of the fibers by the aqueous gelling composition.

EXAMPLES

The following non-limiting examples are intended to illustrate the synthesis of new biocompatible crosslinked polymers and their precursors, and their use in making several medical products. Those skilled in the art will

appreciate that modifications can be made to these examples, drawings, illustrations and claims that are intended to fall within the scope of the present invention.

Materials and Equipment

Polyethylene glycol was purchased from various sources such as Shearwater Polymers, Union Carbide, Fluka and Polysciences. Multifunctional hydroxyl and amine terminated polyethylene glycol were purchased from Shearwater Polymers, Dow Chemicals and Texaco. PLURONIC and TETRONIC series polyols were purchased from BASE Corporation. DL-lactide, glycolide, caprolactone and trimethylene carbonate was obtained from commercial sources like Purac, DuPont, Polysciences, Aldrich, Fluka, Medisorb, Wako and Boehringer Ingelheim. N-hydroxysulfosuccinimide was purchased from Pierce AU other reagents, solvents were of reagent grade and were purchased from commercial sources such as Polysciences, Fluka, Aldrich and Sigma. Most of the reagents and solvents were purified and dried using standard laboratory procedures such as described in D. D. Perrin et al., Purification of Laboratory Chemicals (Pergamon Press 1980).

General Analysis

The polymers synthesized according to these examples were chemically analyzed using structure-determining methods such as nuclear (proton and carbon-13) magnetic resonance spectroscopy, infrared spectroscopy. Molecular weights were determined using high pressure liquid chromatography and gel permeation chromatography. Thermal characterization of the polymers, including melting point and glass transition temperatures, were performed using differential scanning calorimetric analysis. Aqueous solution properties such as micelle and gel formation was determined using fluorescence spectroscopy, UV-visible spectroscopy and laser light scattering instruments.

In vitro degradation of the polymers was followed gravimetrically at 37° C., in an aqueous buffered medium such as phosphate buffered saline (at pH 7.2). In vivo biocompatibility and degradation life times was assessed by injecting or forming a gelling formulation directly into the peritoneal cavity of a rat or rabbit and observing its degradation over a period of 2 days to 12 months.

Alternatively, the degradation was also assessed by prefabricating a sterile implant, made by a process like solution casting, then surgically implanting the implant within an animal body. The degradation of the implant over time was monitored gravimetrically or by chemical analysis. The biocompatibility of the implant was assessed by standard histological techniques.

Example 1

Synthesis of a Water-soluble Difunctional, Biodegradable Functional Polymer Based on Polyalkylene Oxide Block Copolymer

First, Polyethylene glycol-co-polycaprolactone polyol ("F68C2") was synthesized as follows:

30g of PLURONIC F68 was dried under vacuum at 110° C. for 6 h and then mixed with 1.710 g of caprolactone and 30 mg of stannous 2-ethylhexanoate in a glass sealing tube. The glass tube then was sealed under nitrogen atmosphere and heated to 170° C. and maintained at this temperature for 16 h. The PLURONIC F68-caprolactone polymer was cooled and recovered by breaking the glass sealing tube, and then further purified by several precipitations from a toluene-hexane solvent-nonsolvent system.

The polymer then was dried in vacuum at 40° C. and used immediately in the activation reaction described below:

Reaction with Succinic Anhydride ("11F68C2S"):

30 g of PLURONIC F68-caprolactone copolymer was dissolved in 200 ml dry N,N-dimethyl formamide ("DMF") and 0.845 g of succinic anhydride was added to the reaction mixture. The mixture was heated to 100° C. under a nitrogen atmosphere for 16 h. The solution then was cooled and added to 4000 ml hexane to precipitate the carboxyl terminated polymer. It was further purified by repeated (3 times) precipitation from a toluene-hexane solvent-nonsolvent system. The polymer was dried under vacuum at 40° C.

This polymer was immediately used in activation reaction described below:

Activation of Carboxyl Groups with N-hydroxysuccinimide ("F68C2SSNHS"):

30 g of PLURONIC F68-caprolactone succinate copolymer was dissolved in 200 ml dry DMF. The solution was cooled to 4° C. and 1.504 g of 1,3-dicyclohexylcarbodiimide ("DCC" I) and 1.583 g of N-hydroxysulfosuccinimide ("SNHS") were added to the reaction mixture. The mixture was stirred at 4° C. for 6 h and then stirred overnight at room temperature under nitrogen atmosphere. Dicyclohexylurea was removed by filtration and the F68C2S-SNHS derivative was isolated by removing the DMF under vacuum and repeated precipitation using a toluene-hexane solvent-nonsolvent system. The product was stored under nitrogen atmosphere at −20° C.

Example 2

Amine Terminated Synthetic Biodegradable Crosslinkable Polymer

Reaction of F68TMC2SSNHS with Lysine.

3.55 g of lysine was dissolved in 200 ml 0.1M borate buffer (pH 8.5). The mixture was cooled to 0° C. in ice bath and 10 g of F68C2SSNHS were added to the mixture. The mixture was stirred for 6 h at room temperature and lyophilized. The lyophilized powder was dissolved in 30 ml toluene and filtered. The filtrate was added to 4000 ml cold diethyl ether. The precipitated amine terminated polymer was recovered by filtration and dried under vacuum. The polymer was stored under argon at −20° C.

Example 3

Synthesis of Carboxyl Terminated Oligolactic Acid Polymer Activated with N-hydroxysulfosuccinimide

Synthesis of difunctional oligolactate with terminal carboxyl acid end-groups activated with N-hydroxysulfosuccinimide groups.

Part 1: Synthesis of Oligomeric Poly(lactic acid) with Terminal Carboxyl Acid Groups ("PLA-S").

In a 250 ml 3 neck flask equipped with mechanical stirrer, nitrogen inlet and distillation condenser, 2 grams of succinic acid and 34.1 ml 1N HCl and 3.83 g L-lactic acid, sodium salt were charged. The flask was then immersed in a silicone oil bath maintained at 150° C. Most of the water from the reaction mixture was removed over period of 5 hours by distillation. The remaining water was removed by heating the reaction mixture under vacuum at 180° C. for 15 h. The reaction mixture was cooled and lyophilized at 0° C. to remove traces of water. The product was isolated by dis-

solving in toluene and precipitating in hexane. The precipitated polymer was isolated by filtration and dried in vacuum for 48 h at 60° C.

Part 2: Activation of Terminal Groups with N-hydroxysulfosuccinimide Group:

A 3 necked flask equipped with magnetic stirrer and nitrogen inlet was charged with 2 g of PLA-S copolymer and 20 ml DMF. The solution was cooled 4° C. and 3.657 g of N-hydroxysulfosuccinimide and 3.657 g of 1,3-dicyclohexyl carbodiimide were added to the reaction mixture. The mixture was stirred at 4° C. for 6 h and overnight at room temperature under nitrogen atmosphere. Dicyclohexylurea was removed by filtration and SNHS derivative was by isolated by removing the DMF under vacuum and repeated precipitation using toluene-hexane solvent-nonsolvent system. The product was stored under nitrogen atmosphere at 4° C.

Example 4

Preparation of Polyethylene Glycol Based Tetrafunctional Crosslinker.

Part 1: Synthesis of Tetrafunctional Polyethylene Glycol-co-polyglycolate Copolymer ("4PEG2KG"):

30 grams of 4 arm polyethylene glycol, molecular weight 2000 ("4PEG2K") was dried at 100° C. for 16 hours prior to use. 30 grams 4PEG2K. 7.66 g of glycolide and 25 mg of stannous 2-ethylhexanoate were charged into a 3 necked flask equipped with a Teflon coated magnetic stirring needle. The flask was then immersed into silicone oil bath maintained at 160° C. The polymerization reaction was carried out for 16 h under nitrogen atmosphere. At the end of the reaction, the reaction mixture was dissolved in 100 ml toluene. The hydroxy terminated glycolate copolymer was isolated by pouring the toluene solution in 4000 ml cold hexane. It was further purified by repeated dissolution-precipitation process from toluene-hexane solvent-nonsolvent system and dried under vacuum at 60° C. It then was immediately used for end capping reaction mentioned below:

Part 2: Conversion of Hydroxyl Groups into Carboxylic Groups ("4PEG2KGS") and SNHS Ester.

30 g of 4PEG2KG copolymer was dissolved in 150 ml dry pyridine. 8.72 g of succinic anhydride was added to it and the solution was refluxed for 2 h under nitrogen atmosphere. The polymer was isolated by pouring the cold pyridine solution to 4000 ml hexane. The acid terminated polymer ("4PEG2KGS") was used in SNHS activation reaction. Briefly, to a solution of 30 g of 4PEG2KGS in 300 ml dry methylene chloride were added 10.58 g of SNHS and 10.05 g DCC. The reaction mixture was stirred overnight under nitrogen atmosphere. Dicyclohexylurea was removed by filtration. The filtrate was evaporated and the residue obtained was redissolved in 100 ml toluene. The toluene solution was precipitated in 2000 ml hexane. The SNHS activated polymer was stored under nitrogen atmosphere until further use.

Example 5

Sulfonyl Chloride Activated Crosslinkers

Activation of tetrafunctional polyethylene glycol-co-polyglycolate copolymer ("4PEG2KGS") with tresyl chloride.

30 g of 4PEG2KG was dissolved in 10 ml dry benzene. The solution was cooled to 0° C. and 5.92 g of triethyl amine and 10.70 g tresyl chloride were added under nitrogen atmosphere. After refluxing for 3 h under nitrogen atmosphere, the reaction mixture was cooled and filtered to remove triethylamine hydrochloride. The filtrate was poured into 3000 ml hexane to precipitate the activated polymer. The residue was redissolved in THF and filtered over neutral alumina to remove traces of triethylamine hydrochloride. The polymer was recovered by adding the THF solution to 3000 ml diethyl ether and stored under nitrogen atmosphere.

Example 6

Synthesis of Multifunctional Oligopolycaprolactone Terminated with SNHS

Part 1: Synthesis of Polycaprolactone ("PCL1").

2.00 g of glycerol, 8.17 g of caprolactone and 50 mg of stannous 2-ethylhexanoate were charged into 100 ml Pyrex pressure sealing tube. The tube was frozen in liquid nitrogen and connected to vacuum line for 10 minutes. The tube then was connected to argon gas line and sealed under argon. The sealed reaction mixture then was immersed in oil bath maintained at 160° C. and polymerization was carried out for 16 h at 160° C. The polymer was recovered by dissolving it in 30 ml toluene and precipitating in 2000 ml cold hexane. The precipitated liquid oligomer was recovered and dried under vacuum for 1 day at 60° C.

Part 2: End-capping of PCL1 with Succinic Anhydride ("PCL-S"):

10 g of PCL1 was dissolved in 150 ml dry benzene. About 50 ml of benzene was distilled to remove traces of water from the reaction mixture. The solution was cooled to 30° C. To this warm solution, 6.67 g of triethyl amine and 7.86 g of succinic anhydride were added. The reaction mixture was then refluxed for 6 h and concentrated by distillation under vacuum. The product was recovered by adding the filtrate to 2000 ml cold dry hexane.

Part 3: Activation of PCL-S with SNHS:

PCL1-succinate (5.0 g) was dissolved in 10 ml of anhydrous methylene chloride, cooled to 0° C. and 7.82 g of N-hydroxysulfosuccinimide and 7.42 N,N-dicyclohexylcarbodiimide were added under stirring. After stirring the mixture overnight, the precipitated dicyclohexylurea was removed by filtration and the solution was concentrated by removing solvent. The ^{1}H-NMR spectrum showed succinimide singlet at 2.80 ppm (2H).

Example 7

Preparation of Polyethylene Glycol-co-polytrimethylene Carbonate Copolymer Terminated with N-hydroxysuccinimide

Preparation of tetrafunctional polyethylene glycol-co-polytrimethylene carbonate copolymer ("4PEG10KTMC2").

30 g of tetrahydroxy polyethylene glycol, molecular weight 10000, was dried under vacuum at 90–100° C. in a glass sealing tube. The tube then was cooled and transferred inside an air bag where 2.45 g of trimethylene carbonate and 20 mg of stannous octoate were added to the tube. The glass tube was then sealed under vacuum and heated with stirring at 155° C. and maintained at this temperature for 16 h. The polyethylene glycol-co-polytrimethylene carbonate polymer was cooled and recovered by breaking the glass sealing tube. It was further purified by several precipitations from toluene-hexane solvent-nonsolvent system.

Part 2: Synthesis of Glutarate Derivative of 4PEG10KTMC2 ("4PEG10KTMC2G"):

10 g of 4PEG10KTMC was dissolved in 120 ml dry toluene. About 50 ml of toluene was distilled to remove traces of water from the reaction mixture. The warm solution was cooled to 60° C. To this solution, 1.23 g of triethyl amine and 1.40 g of glutaric anhydride were added. The reaction mixture was heated to 60° C. for 1 h and filtered. The product was recovered by adding the filtrate to 2000 ml cold dry hexane.

Part 3: Activation of terminal carboxyl groups using N-hydroxysuccinimide ("4PEG10KTMC2GNHS"):

30 g of 4PEG10KTMC2G was dissolved in 100 ml of dry DMF and 1.53 g of N-hydroxysuccinimide and 5 g molecular sieves 3 Å were added. 1.28 g of DCC dissolved in 5 ml dry DMF was added dropwise and the reaction mixture was kept at room temperature for 24 h under nitrogen atmosphere. The mixture was diluted with 50 ml cold benzene and precipitated using cold hexane. The precipitate was collected on a sintered glass filter with suction. The dissolution and precipitation procedure was then repeated three times, using toluene-diethyl ether as solvent-nonsolvent system and dried under vacuum. The product was stored under nitrogen atmosphere at −20° C. until further use.

Example 8

Succinated Polyhydroxy Compounds Activated with N-hydroxysulfosuccinimide ES

10 g of erythritol was dissolved in 200 ml dry toluene. About 50 ml of toluene was distilled to remove traces of water from the erythritol. The solution was cooled to 50–60° C. and 20 ml pyridine and 8.58 g of succinic anhydride were added to the solution. The reaction mixture was then refluxed for 3 h and unreacted pyridine and toluene were evaporated to dryness under reduced pressure. The residue was used in activation reaction.

Part 2: Activation of ES with SNHS:

Erythritol-succinate (ES, 2.0 g) was dissolved in 10 ml of anhydrous dimethyl formamide ("DMF"), cooled to 0° C. and 3.47 g of N-hydroxysulfosuccinimide and 3.30 N,N-dicyclohexylcarbodiimide were added under stirring. After stirring the mixture overnight, the precipitated dicyclohexylurea was removed by filtration and the solution was concentrated by removing solvent. It was further purified by column chromatography.

Example 9

Preparation of Synthetic Crosslinked Biodegradable Gels

1.57 g (0.8 mM) of 4 arm amine terminated polyethylene glycol molecular weight 2000 was dissolved in 10 ml 0.1 M sodium borate buffer at pH 9.5 2 g of 4 arm SNHS activated 4PEG2KGS polymer (molecular weight 2500) was dissolved in phosphate buffered saline. These two solutions were mixed to produce a crosslinked gel. In another variation of this method, the 4PEG2KGS polymer solid was directly added to the amine terminated polymer solution to produce a crosslinked polymer.

In another variation, a crosslinker consisting of an equimolar solution of dilysine can be used in place of the 4 arm PEG amine solution to form a hydrogel. Gelation was seen to occur within 10 seconds of mixing the two solutions. Similarly, other crosslinkers described in examples 1 to 7 may be reacted in molar equivalent proportions with other amine terminated polymers such as albumin or amine terminated biodegradable polymers similar to described in Example 2. The preferred compositions for making biodegradable hydrogels were described in Table 2. The amine terminated polymer solution described above was added with 0.1% of F D and C blue or indigo dye prior to crosslinking reaction. The addition of dye allows the preparation of colored gels.

Example 10

Preparation of Composite Synthetic Crosslinked Colored Biodegradable Gels

3 grams of bovine serum albumin was dissolved in 3 ml of phosphate buffered solution. Commercial sutures based on synthetic biodegradable polymers, such as Vicryl was cut/ground into several small pieces (size less than 1 mm) using cryogenic grinding. These colored suture particles (approximately 100 mg) were mixed with the albumin solution to form a suspension. 100 mg of crosslinker such as 4PEG10KTMC2GNHS was mixed with 0.2 ml of albumin suspension. This viscous solution then was mixed with 40 mg of triethanol amine (buffering agent). The addition of triethanol amine gels the solution in 60 seconds. The colored suture particles entrapped in the crosslinked gel help to visualize the gel especially when under laparoscopic conditions and also acts to strengthen the hydrogel as a reinforcing agent. The suture particles in above examples can be replaced with biodegradable microparticles loaded with drugs or bioactive compounds.

Example 11

Formulation of SG-PEG with Di-lysine

A four arm PEG with SG end groups (Shearwater Polymers, approx. 9,100 g/mol, 0.704 grams, 6.5×10^{-5} moles) was dissolved in 2.96 g 0.01M pH 4.0 phosphate buffer (19.2% solids). Di-lysine (Sigma, 347.3 g/mol, 0.03 grams, 8.7×10^{-5} moles) was dissolved in 3.64 grams of 0.1M pH 9.5 borate buffer (0.8% solids). On combination of the two solutions, the percent solids was 10%. The di-lysine has 3 amine groups. The SG-PEG has 4 NHS groups. After correction for the less than 100% degree of substitution on the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

Example 12

Formulation of SG-PEG with Tri-lysine

A four arm PEG with SG end groups (Shearwater Polymers, approx. 9,100 g/mol, 0.675 grams, 6.2×10^{-5} moles) was dissolved in 2.82 g 0.01M pH 4.0 phosphate buffer (19.3% solids). Tri-lysine (Sigma, 402.5 g/mol, 0.025 grams, 6.2×10^{-5} moles) was dissolved in 3.47 grams of 0.1M pH 9.5 borate buffer (0.7% solids). On combination of the two solutions, the percent solids was 10%. The tri-lysine has 4 amine groups. The SG-PEG has 4 NHS groups. After correction for the less than 100% degree of substitution on the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

Example 13

Formulation of SG-PEG with Tetra-lysine

A four arm PEG with SG end groups (Shearwater Polymers, approx. 9,100 g/mol, 0.640 grams, 5.9×10^{-5} moles) was dissolved in 2.68 g 0.01M pH 4.0 phosphate buffer (19.2% solids). Tetra-lysine (Sigma, 530.7 g/mol, 0.025 grams, 4.7×10-' moles) was dissolved in 3.30 grams of 0.1M pH 9.5 borate buffer (0.8% solids). On combination of the two solutions, the percent solids was 10%. The tetra-lysine has 5 amine groups. The SG-PEG has 4 NHS groups. After correction for the less than 100% degree of substitution on the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

Example 14

Gel Time Measurement

The amine solution (100 μL) was aliquotted into a 100×13 test tube. A flea-stirbar (7×2 mm, Fisher Scientific p/n 58948–976) was placed in the test tube. The test tube was held stationary over a digital magnetic stirrer (VWR Series 400S Stirrer) set at 300 rpm. A 1 cc tuberculin syringe (Becton Dickinson, p/n BD309602) was filled with 100 μL of the ester solution. The syringe was inserted up to the flanges so that the distal end was just over the amine solution. Simultaneously the plunger was depressed and a stop watch started. When the solution solidifies sufficiently so that the stir bar stops spinning, the stop watch was stopped. Each solution was measured in triplicate and the mean ±1 standard deviation was plotted. Results for the formulations of examples 1, 2 and 3 are shown in FIG. 11.

Example 15

Change in Gel Time as a Function of Ester Solution Age

An important characteristic of these systems is the loss in reactivity over time from reconstitution of the ester solution. This loss in reactivity occurs due to hydrolysis of the N-hydroxysuccinimidyl ester, before the activated molecule can combine with its respective nucleophilic functional group. The loss of reactivity was characterized by measuring the change in gel time as a function of time from reconstitution of the NHS ester solution. The gel time was measured periodically. The NHS ester solution was stored at ambient conditions during this measurement. Results for the solutions described in Examples 11, 12 and 13 are shown in FIG. **12**.

Example 16

Gel Formation at Different Percent Solids from 4 Arm CM-HBA-NS PEG and Lys-Lys

Using the gel time method described in Example 13, five different gel compositions were made using carboxymethyl hydroxybutyrate-hydroxysuccinimide end-capped 4 arm PEG (CM-HBA) (Shearwater Polymers) and di-lysine (Sigma). The formulations are listed below in Table 3.

TABLE 3

Conc. (%)	CM-HBA (g)	Phosphate (g)	Lys-Lys (g)	Borate (g)
8.5	0.2469	1.264	0.01	1.5012
10	0.2904	1.2209	0.012	1.4994
12.5	0.363	1.1483	0.015	1.4964
15	0.4356	1.0757	0.018	1.4936
20	0.5808	0.9305	0.024	1.4876

The formulations were adjusted to give a 1 to 1 ratio of electrophilic functional end groups on the CM-HBA (4) to nucleophilic reactive groups on the di-lysine ("Lys-Lys")(3). The CM-HBA quantities were dissolved in 0.01M pH 5.0 phosphate buffer. The di-lysine was dissolved in 0.1M pH 11 borate buffer. Gel time results are shown in FIG. **13**. This data also shows that the higher percent solids solutions also are the most stable with respect to retention of speed of reaction.

Example 17

Degradation of Hydrogels

Hydrogel plugs made during the gel time measurements of Example 14 were placed in approximately 25 mL 0.1M phosphate buffered saline at pH 7.4 in 50 mL Falcon tubes and placed in a constant temperature bath at 37° C. The hydrogel plugs were observed visually at periodic intervals and the time of gel disappearance noted. The data are plotted in FIG. **14**.

Example 18

Precursor Spray Procedure to Form a 7.5% Solids Hydrogel from 4 Arm SG and Dilysine

An ethylene oxide sterilized air assisted sprayer was used in conjunction with aqueous solutions of polymerizable monomers. Solution 1 consisted of a 14.4% solution of 4 arm SG (MW 10,000 purchased from Shearwater Polymers) dissolved in 0.01M phosphate buffer at pH 4.0 and was sterile filtered (Pall Gelman syringe filter, p/n 4905) and drawn up in a sterile 5 cc syringe. Solution 2 consisted of a 1.2% solution of a dilysine (purchased from Sigma Chemicals) dissolved in 0.1M borate buffer at pH 11 with 0.5 mg/mL methylene blue for visualization and was also sterile filtered and drawn up in a sterile 5 cc syringe. These solutions, when combined 1:1 on a volumetric basis, resulted in a 1:1 ratio of NHS ester to amine end group. The final % solids after combination was 7.5%. The two syringes were individually loaded in the two separate receptacles through a luer-lok type of linkage. Airflow from a regulated source of compressed air (an air compressor such as those commercially available for airbrushes) was connected to the device using a piece of Tygon tube. On compressing the syringe plungers a steady spray of the two liquid components was observed. When this spray was directed to a piece of tissue (rat cecum) a hydrogel coating was observed to form on the surface of the tissue. This hydrogel coating was rinsed with saline (the hydrogel coating is resistant to rinsing) and was observed to be well adherent to the tissue surface. Within a short period of time (less than a minute) an area of 10 cm×5 cm could be coated with ease.

Example 19

Precursor Spray Procedure to Form a 12.5% Solids Hydrogel from 4 Arm CM and Dilysine

A hydrogel barrier film made from 4 arm CM-HBA NS (MW 10,000 purchased from Shearwater Polymers), and dilysine was similarly prepared and sprayed as described in Example 18. In the present example the 4 arm CM solution was made up to 24.0% solids and the dilysine solution was made up to 1.0% solids such that on combination in an equal volume delivery system a 1:1 ratio of NHS to amine end groups results, giving a final % solids of 12.5%.

Example 20

Spray Application of Crosslinker and Polymer to from Crosslinked Film

Two solutions (component A and component B) were prepared. Component A consisted of dilysine in 0.1M borate buffer, pH 9.5. Component B consisted of either 4 arm SG-PEG or 4 arm CM-HBA-NS in 0.01M phosphate buffer, pH 4.0 These solutions were prepared such that the amine to ester stoichiometric ratio was 1:1 and the final total solution concentration was 7.5% or 12.5%, respectively.

A FIBRIJECT™ (Micromedics, Inc.) 5 cc syringe holder and cap was used, preloaded with 5 cc of each solution and attached to a dual barrel atomizing sprayer. The sprayer has two hubs for the syringes to connect to allowing the two fluids to be advanced through two separate lumens over any preset distance. A third hub exists for the application of the atomizing gas. Air was used in this example. The distal tip of the sprayer contains a chamber where the gas expands out of an introduction tube, then flows past the two polymer solution nozzles in an annular space around each. The gas is accelerated in the annular spaces using a flow rate suitable for the complete atomization of the two fluid streams (~2L/min.). Two overlapping spray cones are thus formed allowing for well mixed, thin, uniform coatings to be applied to surfaces.

Example 21

Adhesion Prevention in Rat Cecum Model

Surgical Procedure

Male Sprague Dawley rats (250–300 grains,) were anesthetized with an intramuscular 4ml/kg "cocktail" of KETAMINE (25 mg/ml), XYLAZTNE (1.3mg/mL) and ACEPROMAZINE (0.33 mg/mL). The abdominal area was shaved and prepped for aseptic surgery. A midline incision was made to expose the abdominal contents. The cecum was identified and location within the abdomen was noted. The cecum was pulled out of the abdomen and the surface of one side was abraded using dry sterile gauze. A technique of abrading one area by stroking the surface 12 times with the gauze was used. The cecal arterial supply was interrupted using bipolar coagulation along the entire surface area of the damaged cecum.

The opposing abdominal sidewall which lays in proximity to the damaged cecal surface was deperitonealized with a scalpel blade and the underlying muscle layer was scraped to the point of hemorrhaging.

The cecum was sprayed with either the SG-PEG system or the CM-HBA-NS system using the air assisted spray method described in the preceding example. The cecum was placed with the damaged (ischemic area) side up opposite the damaged side wall. Active bleeding was controlled before closing. The peritoneum and muscle wall was closed with 3-0 nylon and the skin was closed with 4-0 silk. Rats were returned to their cages for one to two weeks at which time evaluation of the adhesion between the side wall and cecum was noted. The rats were killed at 10 days and the tenacity and extent of adhesion was evaluated. The results are summarized in Table 4.

TABLE 4

Rat #	Material Applied	Reference Example	Finding on Day 10
403	7.5% 4aSG with Lys-Lys w/MB	Example 18	Small amount of gel present on cecum. No adhesions from cecum to sidewall. No gel on sidewall
404	7.5% 4aSG with Lys-Lys w/MB	Example 18	Some mesentery stuck to cecum. No gel. No adhesions.
405	7.5% 4aSG with Lys-Lys w/MB	Example 18	Small amount of gel present on cecum. Some mesentery stuck to cecum and sidewall. Some gel between mesentery and cecum where stuck. No adhesions.
406	12.5% 4aCM with Lys-Lys w/MB	Example 19	No gel present. No adhesions.
407	12.5% 4aCM with Lys-Lys w/MB	Example 19	No gel on cecum or sidewall. No adhesions.
408	12.5% 4aCM with Lys-Lys w/MB	Example 19	Rat died post-op (anesthesia overdose).

Example 22

This example is directed to concentrations of coloring agent for use in an in situ crosslinked hydrogel coating. A 140 mg amount of four-arm primary amine terminated polyethylene glycol molecule with a molecular weight of approximately 10,000 was dissolved in sodium borate buffer pH 9.5. An 84 mg amount of four arm NHS activated polyethylene glycol polymer (SPA-3400, Shearwater Corp., Huntsville, Ala., molecular weight approximately 3400) was dissolved in pH 5.0 acetate buffer. Methylene Blue was added to the borate buffered solutions at concentrations of 0.1, 0.5, and 1.0 mg/ml.

A standard laparoscopic sprayer was used in a laparoscopic trainer to spray the surfaces of pieces of lunch meat with an approximately 1:1 mixture of the solutions. The mixture formed a gel in about 3–6 seconds on the surfaces. The sprayed gel was observed through a 10 mm laparoscope and videotaped. The tapes were reviewed to assess the effect of the coloring agent. The 0.5 mg/ml and 1.0 mg/ml solutions of coloring agent created a gel that was readily observable and similar in visibility. The 0.1 mg/ml solution of coloring agent created a gel that was light in color and more difficult to observe compared to the other solutions. Many previous experiments had already shown that gels with no coloring agents were very difficult to observe visually. Control experiments performed without the presence of methylene blue showed that the methylene blue did not affect gel times under these conditions.

A similar experiment was performed using 4 arm NHS polyethylene glycol (molecular weight 10,000) mixed with an equimolar concentration of a multiarm amine-terminated polyethylene glycol (molecular weight 20,000). FD&C Blue #2 dye was present in the resultant hydrogel at a concen-

tration of 0.05, 0.1, 0.25, 0.5, 1.0, and 2.5 mg/ml. The hydrogel was applied at a thickness of about 1.0 mm and observed by two independent observers and the ability to observe the gel was rated as adequate or inadequate. The results showed that good visualization of the hydrogel could be obtained at concentrations of at least 0.25 mg/ml FD&C Blue #2.

Example 23

This example was directed to the evaluation of the stability of colorants in solution. An 0.5 mg/ml amount of FD&C BLUE #2 dye (also called indigo carmine) was dissolved in 0.1 M sodium borate decahydrate pH 10 buffer, in deionized water, and in 0.01 M sodium phosphate buffer pH 4.0. Solutions were stored for up to 48 hours at 4° C., 25° C., and 40° C. The dye appeared visually to be stable in solubility and color in the distilled water and phosphate buffer solutions although the dye changed the pH of the phosphate buffer from 4 to 6.9. The dye changed color in the borate solution. FD&C blue #2 and Methylene blue were observed to be not completely soluble so that their concentration is significant. FD&C Blue #2 was soluble at less than 2.5 mg/ml but its maximum solubility was not ascertained.

Example 24

This experiment was directed to the effect of coloring agents on gelation times. A 4 arm NHS polyethylene glycol (molecular weight 10,000) solution was mixed with an equimolar concentration of a multiarm amine. Both an amine terminated polyethylene glycol (molecular weight 20,000) was evaluated as well as dilysine. Visualization agent was mixed with the buffer used to reconstitute the amine and was present in the resultant hydrogel at a concentration of 12.5 mg/ml. Gel time tests were performed in triplicate. Gel time was measured immediately on reconstitution of the ester (time zero) and 1.5 hours later. The mean gelation times in seconds±standard deviation were: FD&C Blue #1 gelation time 1.57±0.12 time zero compared to 2.2±0.05 after 1.5 hours; FD&C Blue #2 gelation time 1.51±0.12 time zero compared to 2.08±0.09 after 1.5 hours; Methylene Blue gelation time 1.67±0.28 at time zero compared to 1.97±0.12 after 1.5 hours; No visualization agent gelation time 1.39±0.02 compared to 1.78±0.13 after 1.5 hours. These visualization agents did not cause an unacceptable change in gelation times.

While preferred illustrative embodiments of the invention are described above, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications which fall within the true spirit and scope of the invention.

What is claimed is:

1. A method of preparing a composition suitable to coat a tissue of a patient, the method comprising:

mixing reactive precursor species comprising nucleophilic functional groups, reactive precursor species comprising electrophilic functional groups, and a visualization agent such that the nucleophilic functional groups and electrophilic functional groups crosslink after contact with the tissue to form a hydrogel having an interior and an exterior, with the exterior having at least one substrate coating surface and the visualization agent being at least partially disposed within the interior and reflecting or emitting light at a wavelength detectable to a human eye to thereby provide a means for visualization of the coating by a human eye.

2. The method of claim **1**, wherein the hydrogel comprises crosslinked polymers that are selected from the group consisting of collagen, fibrinogen, albumin, and fibrin.

3. The method of claim **1**, wherein the hydrogel is made of synthetic materials.

4. The method of claim **1**, wherein the hydrogel is hydrolytically biodegradable.

5. The method of claim **1**, wherein the hydrogel comprises covalently crosslinked hydrophilic polymers.

6. The polymeric coating method of claim **1**, wherein the visualization agent is chosen from the group consisting of FD&C Blue #1, FD&C Blue #2, methylene blue, indocyanine green, visualization agents that provide a blue color, and visualization agents that provide a green color.

7. The method of claim 1, wherein the visualization agent is covalently linked to the hydrogel.

8. The method of claim **1**, wherein the hydrogel comprises a biologically active agent.

9. The method of claim 1, wherein the hydrogel forms within 60 seconds after contact with the substrate.

10. The method of claim **1**, wherein the hydrogel forms within 5 seconds after contact with the substrate.

11. The method of claim **1**, wherein the biodegradable hydrogel is adherent to the tissue.

12. A hydrogel composition adapted for use with a tissue of a patient, the composition being made by the process of claim **11**.

13. The method of claim **1**, further comprising:

applying the hydrogel onto the tissue until an average thickness is reached in which the color of the hydrogel indicates that a predetermined thickness of hydrogel has been deposited on the tissue.

14. The method of claim **13**, comprising choosing the predetermined thickness to be about 0.5 to about 4.0 mm.

15. The method of claim **13**, comprising choosing at least one of the reactive precursor species to have a hydrolytically biodegradable portion such that the hydrogel is biodegradable.

16. A method for formulating a polymer composition that crosslinks to form a hydrogel, the method comprising selecting a concentration of visualization agent for the polymer composition such that the visualization agent causes a visually observable change that indicates that a crosslinked hydrogel having a predetermined thickness has been formed on the tissue of a patient wherein the polymer composition comprises electrophilic functional groups and nucleophilic functional groups that crosslink to each other.

17. The method of claim **16**, wherein the predetermined thickness is from about 0.1 mm to about 10.0 mm.

18. The method of claim **16**, wherein the observable change is not being able to see a substrate through the polymer composition.

19. The method of claim **16**, wherein the observable change is not being able to see patterns in a substrate surface through the polymer composition.

20. The method of claim **16**, wherein the polymer composition crosslinks to form a hydrogel within about 60 seconds after being applied to a substrate.

21. The method of claim **16**, further comprising mixing the visualization agent at a selected concentration with reactive precursor species.

22. The method of claim **16**, further comprising a biologically active agent.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,009,034 B2
APPLICATION NO. : 10/010715
DATED : March 7, 2006
INVENTOR(S) : Chandrashekhar P. Pathak, Amarpreet S. Sawhney and Peter Edelman

It is certified that error appears in the above--identified patent and that said Letters Patent is hereby corrected as shown below:

On Title page Item [56]
Page 1, Col. 2, Other Publications, delete "MD.,", and insert --M.D.,--.
In drawing Sheet 3 of 10, delete "FIQ" and insert --FIG--.
Col. 11, Line 67, insert --; -- before "the".
Col. 12, Line 27, delete "copolyiner" and insert --copolymer--.
Col. 14, Line 21, delete "ate" and insert --are--.
Col. 14, Line 14, delete "copolyiners" and insert --copolymers--.
Col. 14, Line 52, delete "NTIS" and insert --NHS--.
Col. 14, Line 53, delete "tenninal" and insert --terminal--.
Col. 14, Line 56, delete "N-bydroxysuccinimide" and insert --N-hydroxysuccinimide--.
Col. 16, Table 1, Line 64, col. 2, delete "tetrafuncational" and insert --tetrafunctional--.
Col. 17, Table 1, Line 32, col. 3, delete "oligocaprolatone" and insert --oligocaprolactone--.
Col. 17, Table 1, Line 35, col. 3, delete "hydroxysulfosuccinimi de" and insert --hydroxysulfosuccinimide--.
Col. 17, Table 1, Line 41, col. 3, delete "hydroxysulfosuccinimi de" and insert --hydroxysulfosuccinimide--.
Col. 19, Line 53, delete "2-ethyihexanoate" and insert --2-ethylhexanoate--.
Col. 19, Line 63, delete "tenninated" and insert --terminated--.
Col. 26, Line 1, after "like" insert --"--.
Col. 28, Line 49, delete "aqaeous" and insert --aqueous--.
Col. 29, Line 10, delete "BASE" and insert --BASF--.
Col. 29, Line 15, delete "AU" and insert --. All--.
Col. 37, Line 48, delete "grains,)" and insert --grams,)--.
Col. 37, Line 50, delete "XYLAZTNE" and insert --XYLAZINE--.
Col. 40, Line 12, In Claim 6, after "The" delete "polymeric coating".

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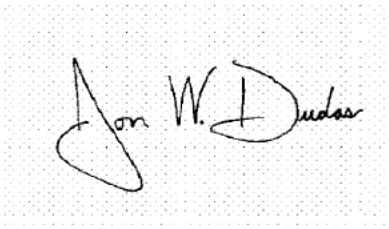
It is certified that error appears in the above--identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 40, Line 47, In Claim 16, after "patient" insert --,--.

Signed and Sealed this

Seventeenth Day of April, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office